SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 2, 5-DISUBSTITUTED BENZIMIDAZOLE DERIVATIVES

Dissertation Submitted to

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Chennai – 600 032.

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

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(Accredited by “NAAC” with a CGPA OF 2.74 on a four point scale at “B”-Grade)

Melmaruvathur - 603 319

May - 2012

CERTIFICATE

This is to certify that the research work entitled **"SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 2,5-DISUBSTITUTED BENZIMIDAZOLE DERIVATIVES"** submitted to The Tamil Nadu Dr. M.G.R Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Chemistry) was carried out by **M. YOKESH KUMAR (Register No: 26106040)** in the Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct guidance and supervision during the academic year 2011-2012.

Place: Melmaruvathur

Date:

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Place: Melmaruvathur

Date:

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M.YOKESH KUMAR.

DeDicateD

to my

brother &

my parents

CONTENTS

S. No	Title	Page. No.
1.	**INTRODUCTION**	1
2.	**LITERATURE REVIEW**	8-19
3.	**AIM AND PLAN OF WORK**	20-21
4.	**EXPERIMENTAL**	22
4.1	Material and Instruments	22-23
4.2	Methodology	24
4.3	Synthesis of compounds	25-37
5.	**BIOLOGICAL SCREENING**	38
5.1	Evaluation of Antibacterial activity	38
5.2	Evaluation of Antifungal activity	39
6.	**RESULTS AND DISCUSSION**	40
6.1	Synthetic Scheme	40-44
6.2	Interpretation of spectral data of synthesized compounds	45-122
6.3(a)	Physical and Analytical data of the synthesized compounds	123-128
(b)	Comparative physiochemical data of conventional and microwave assisted synthesized compounds	129
6.4	Screening of Antimicrobial activity	130-135
7.	**SUMMARY AND CONCLUSION**	136-139
8.	**BIBLIOGRAPHY**	140-144
9.	**ANNEXURE – I**	145

LIST OF ABBREVIATIONS

%	Percentage
µg	Microgram
ATCC	American type culture collection
DMF	Dimethyl Formamide
DMSO	Dimethyl Sulphoxide
DMSO - d_6	Deutrated dimethyl sulphoxide
Eg	Example
EtOH	Ethanol
G	Gram
Gram – ve	Gram Negative
Gram +ve	Gram Positive
h	Hours
HCMC	Human cytomegalo virus
HIV	Human immuno defecieny virus
HSV	Herpes simplex virus
IC_{50}	Inhibitory concentration
IR	Infrared Spectroscopy
L	Littre

M	Mole
Min	Minutes
M.P	Melting Point
MASS	Mass spectroscopy
MeOD	Deutrated methanol
MeOH	Methanol
mg	Milligram
MIC	Minimum inhibitory concentration
ml	Milliliter
MLR	Multiple linear regression
NMR	Nuclear Magnetic Resonance
NCTC	National collection type cultures
O	Ortho
^{o} C	Degree Centigrade
PABA	Para amino benzoic acid
PPA	Poly phosphoric acid
ppm	Parts per million
QSAR	Quantitative Structure Activity Relationship
R_f	Retardation factor
Sec	Seconds
TLC	Thin Layer Chromatography

introduction

1. INTRODUCTION

Medicinal chemistry involves the discovery, development, identification and interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the significance of medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds. Medicinal chemistry covers three critical steps.

A discovery step, involves the choice of the therapeutic target (receptor, enzyme, transport group, cellular, or *in vivo* model) and the identification (or discovery) and production of new active substances interacting with the selected target. Such compounds are usually called lead compounds; they can originate from synthetic organic chemistry, from natural sources, or from biotechnological process. Drugs design aims at the development of the drugs with high specificity and therapeutic indeed.

An optimization step, which deals with the improvement of the lead structure. The optimization process takes primarily in to account the increase in potency, selectivity and toxicity. Its characteristics are the establishment and analysis of structure activity relationships, in an ideal context to enable the understanding of the molecular mode of action. However, an assessment of the pharmacokinetic parameters such as absorption, distribution, metabolism, excretion and oral bioavailability is almost systematically practiced at an early stage of the development in order to eliminate unsatisfactory candidates.

A development step, whose purpose is the continuation of the improvement of the pharmacokinetic properties and the fine tuning of the pharmaceutical properties (chemical formulation) of the active substances in order to render them suitable for clinical use. This chemical formulation process can consist in the preparation of better absorbed compounds, of sustained release formulations, and of water soluble derivatives or in the elimination of properties related to the patient's compliance (causticity, irritation, painful injections and undesirable organoleptic properties.)

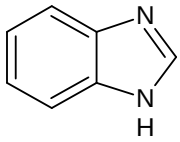
Benzimidazoles are five membered benzoheterocyclic compounds containing two hetero atoms. Both hetero atoms are nitrogen, which are present at non-adjacent position. Benzimidazole derivatives belong to a crucial structural motif that is seen in many pharmaceutically and biologically interesting molecules. Recent publications have been reported to possess a number of significant and diverse biological activities such as fungicide, anti-oxidant, anti-microbial, anthelmintic, anti-cancer, anti-hypertensive, anti-neoplastic, anti-inflammatory, analgesic, anti-protozoal, and anti-hepatitis B virus activity. Some of their analogues show an array of biological activities, including non-nucleoside HIV-1 reverse transcriptase inhibitors and they selective inhibitors of cyclooxygenase Cox-2.

In view of these activities and synthetic importance, benzimidazole core and its various derivatives have long been an area of interest and still continue as an active domain for research and industrial field. These versatile biological significance inspired us to synthesize the 2, 5 di-substituted benzimidazoles.

Traditional synthesis involves the reaction between a O-phenylene diamine and a carboxylic acid under harsh dehydrating reaction conditions. A number of synthetic methods have been developed in recent years to uncover a variety of new reagents for the synthesis of 2-substituted benzimidazole under milder conditions by the addition of lewis acids, inorganic clays and mineral acids. Long reaction time for this reaction has been reduced by the use of microwave heating, both with and without polyphosporic acid.

Benzimidazole (Anonymous http:// www.wikipedia.org)

Structure:



Formula : $C_7H_6N_2$

Molecular weight : 118.17

Toxicity : Oral rat LD50:2910mg/Kg

Synonyms : 1H-Benzimidazole; 1,3-benzodiazole;benzoglyoxaline; 3-azindole; N,N'methylenyl-O-phenylenediamine; 3-azaindole;O-benzimidazole; benzoimidazole; BZI; 1,3diazaindene.

Physical and chemical properties:

Melting point : 176° C

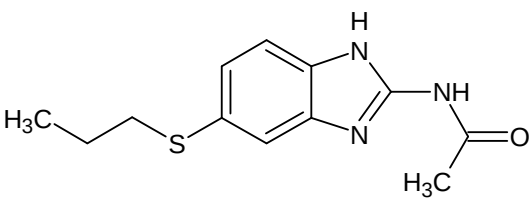
Boiling point	:	360° C
Specific gravity	:	1
Solubility in water	:	slightly soluble
Auto ignition	:	538° C
Stability	:	Stable under normal temperature and condition.

Special interest of researchers triggered by the fact that 5, 6-dimethyl benzimidazole is a component of naturally occurring vitamin $B_{12.}$ A large number of benzimidazole derivatives were shown to exhibit important biological properties such as anti bacterial, anti fungal, anti-helminthic, anti-allergic, anti-neoplastic, local analgesic, antihistaminic, anti-leishmanial, vasodilator, anti-hypertensive, spasmolytic, and anti-ulcer activities. *(Hasan Kucukbay, et al., 2010)*. A large variety of 2-substituted benzimidazoles have been found to posses anti inflammatory, anti-spasmodic, anti histaminic, antimicrobial, anticancer and cyclooxygenase inhibitor activity. *(Gupta S.K et al., 2010)*

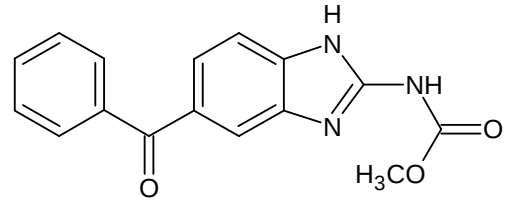
Also, some benzimidazole nucleosides, particularly 5, 6-dichloro benzimidazole-1-β-D-ribofuranoside (DRB) and its 2-substituted derivatives show activity against human cytomegalovirus. It is also known that 5, 6-dinitro benzimidazole can substitute 5, 6-dimethyl benzimidazole in the vitamin B_{12} molecule in *coryne bacterium diphtheria* and 2-trifluoro benzimidazoles are potent (*Zygmunt Kazimierczuk, et al., 2002)*. Benzimidazole derivatives exhibit significant activity against several viruses

such as HIV, herpes (HSV-1), RNA, influenza, and human cytomegalovirus (HCMV). *(Han xiangming et al., 2007)*

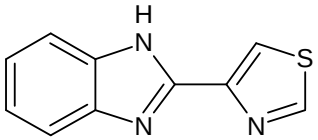
Benzimidazole substituted with a sugar residue at C-2 is potent inhibitors of glycogen phosphorylase and have become the targets for new drug development for treatment of diabetes mellitus. *(Leonard J.T et al., 2007)*



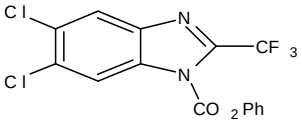
Albendazole (anthelmintic)



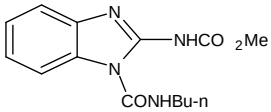
Mebendazole (veterinary anthelmintic)



Thiobendazole (human

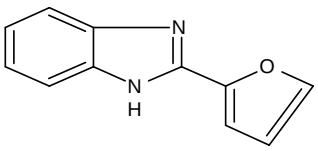


Veterinary anthelmintic)

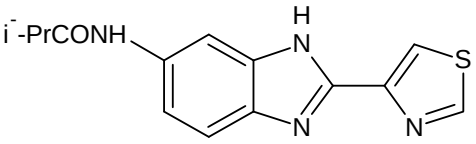


Fenzaflor (acarcide)

Benomyl(fungicide)

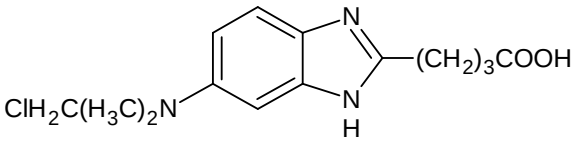


Fuberidazole (fungicide)

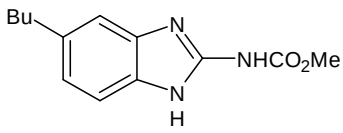


Cambendazole (veterinary anthelmintic)

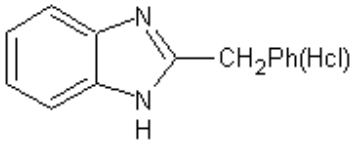
Fig-1: Compounds containing benzimidazole nucleus



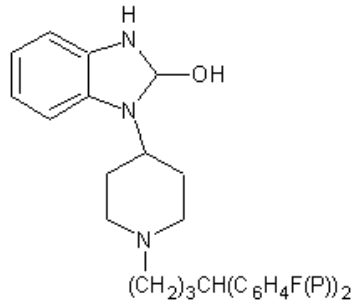
Imet3393 (anti cancer)



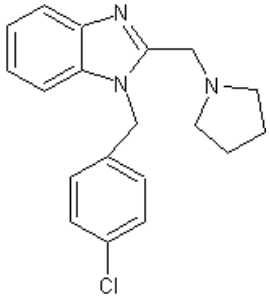
Parbendazole (veterinary anthelmintic)



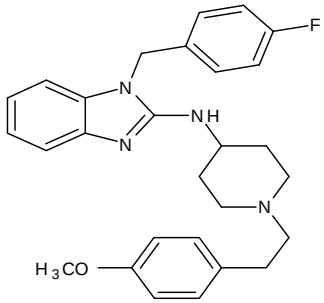
Diabazole (spasmolytic, antihypertensive)



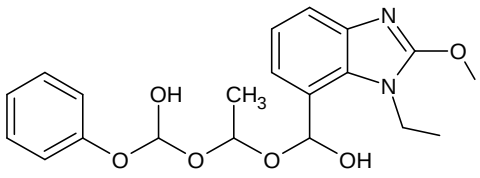
Pimozide (psychopharmacological agent)



Clemizole penicillin (bactericide)

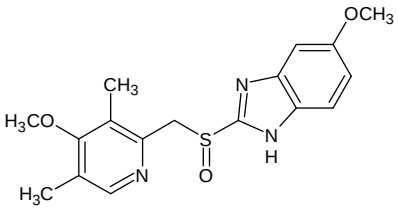


c Astemizole (anti ulcer)

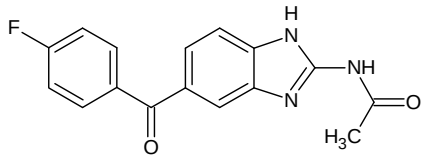


Milfasartan (anti hypertensive)

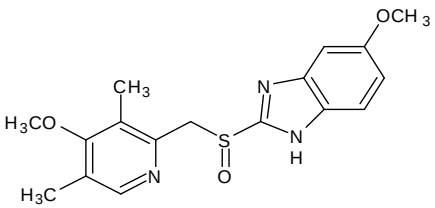
Fig-1: Compounds containing benzimidazole nucleus (contd)



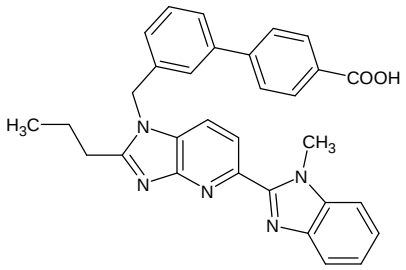
Omeperazole (anti ulcer)



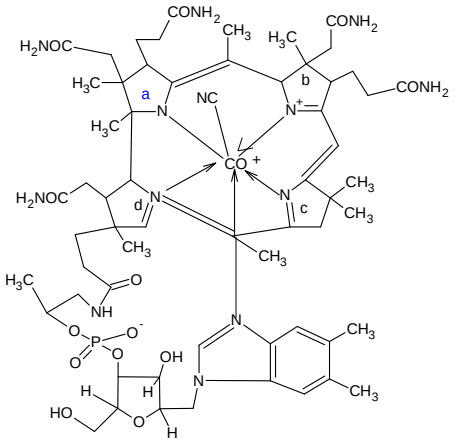
Flubendazole (fungicide)



Lansoperazole (anti ulcer)



Telmisartan (anti hypertensive)



Vitamin-B_{12}

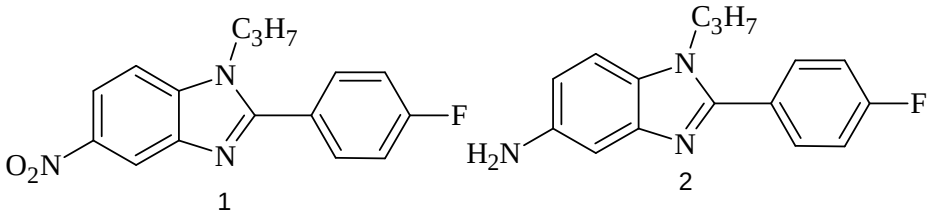
Fig-1: Compounds containing benzimidazole nucleus

Although a variety of benzimidazole derivatives are known, the development of new and convenient strategies to synthesize new biologically active benzimidazole is of considerable interest. *(Gupta S.K etal., 2010)*

LITERATURE REVIEW

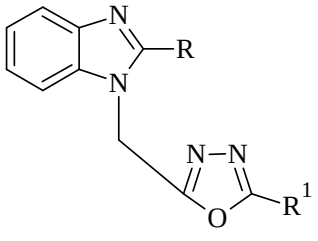
2. LITERATURE REVIEW

2.1) Ayhan Kilcig G. *et al.,* 2006 synthesized some novel benzimidazole derivatives and they were evaluated for their anti-fungal activities against *Candida albicans, Candida glabrata* and *Candida krusei*. Compounds 1 and 2 (Fig-2) possessed good anti-fungal activity comparable to Fluconazole against *Candida albicans*.



(Fig-2)

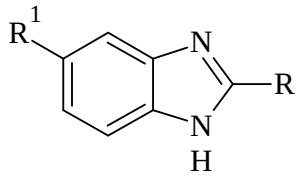
2.2) Ansari K.F. *et al.,* 2009 developed a series of 2-substituted-1-[{(5-substituted alkyl/aryl)-1,3,4-oxadiazol-2-yl}methyl]-1H-benzimidazole derivatives, the synthesized compounds were identified by spectral and elemental methods of analyses. All the synthesized compounds were screened for their anti-microbial activities. All of the derivatives showed good activity towards gram-positive bacteria and negligible activity towards gram-negative bacteria. Some of the synthesized compounds (Fig-3) showed good activity against tested fungi.



(Fig-3)

Code	R	R^1
1	H	2-ClC_6H_4
2	H	4-ClC_6H_4
3	H	2-OHC_6H_4

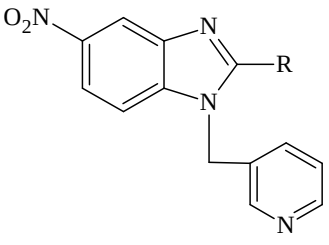
2.3) OztekinAlgul *et al.,* 2007 investigated the structure-activity relationship for some newly synthesized benzimidazole structures and they were synthesized 2-substituted benzimidazole derivatives. The synthesized compounds were examined for anti-bacterial activity against both gram-positive (*Enterococcus faecalis, Staphylococcus epidermis*) and gram-negative (*Escherichia coli, Pseudomonas aeurginosa*) organisms and anti-fungal activity against *Candida albicans, Candida kruesi, Candida glabrata, Candida tropicalis* and *Candida parapsilosis*. All of the compounds (Fig-4) were more active towards bacteria than fungi.



(Fig-4)

Compound	R	R^1
1	CH_2OH	H
2	CH_2OH	CH_3
4	CH_2NH_2	H
5	CH_3	H
6	CH_2SH	H

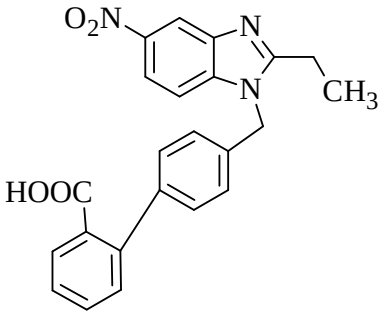
2.4) Jitender Singh *et al.,* 2010 synthesized a series of 1, 2, 5-tri substituted benzimidazole derivatives and the derivatives were examined for anti-convulsant activity. The results of QSAR investigation and the study of various Physiochemical properties indicates that the change in linker position (R) does not change the activity, the compound 6f and 6j (Fig-5) possessed good anti-convulsant activity.



(Fig-5)

Compound	R
6f	H
6j	CH_3

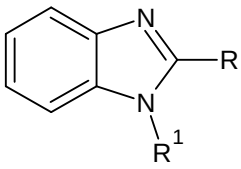
2.5) Jat Rakesh Kumar., *et al* 2006 developed a new series of 5-substituted (amino) -2-phenyl-1-(2-carboxy biphenyl-4-yl) benzimidazole derivatives and they were evaluated for anti-hypertensive activity. From the study it was found that compounds contain ethyl group at 2nd position (Fig-6) gave better result compare to phenyl at position 2.



(Fig-6)

Compound	R	R^1
1	CH_2CH_2COOH	$CH_2C_6H_5$
2	C_6H_4OH	$CH_2C_6H_5$
5	CHOH.CHOH.COOH	$CH_2C_6H_5$
8	CH_2CH_2COOH	ClC_6H_5

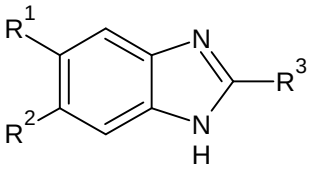
2.6) Ashish Kumar Tewar *et al.,* 2006 synthesized a new series of N-substituted-2-substituted benzimidazole derivatives, 1-benzyl-2-substituted benzimidazole and 1-(p-chlorophenyl)-2-substituted benzimidazole derivatives. The synthesized compounds were tested for their anti-viral activities against *tobacco mosaic virus* and *sun hemp rosette* viruses. The compounds 1, 2, 5 and 8 (Fig-7) were showed significant activity.



(Fig-7)

Code	R	R^1
1	CH_2CH_2COOH	$CH_2C_6H_5$
2	C_6H_4OH	$CH_2C_6H_5$
5	CHOH.CHOH.COOH	$CH_2C_6H_5$
8	CH_2CH_2COOH	ClC_6H_5

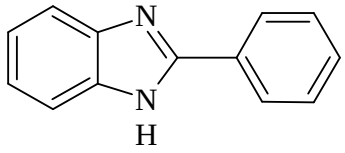
2.7) Goel P.K. *et al*., 2007 investigated the quantitative structure activity relationship (QSAR) of a set of twenty 1H- benzimidazole derivatives with anti-protozoal activity against *Entamoeba histolytica*. The studies used various combinations of thermodynamic, electronic, and spatial descriptors. By assuming the significance of the contributed descriptors for the inhibition of amoebic activity, they were synthesized six new compounds (BZ_1 to BZ_6) (Fig-8) for the better inhibitory activity with less toxicity.



(Fig-8)

Code	R^1	R^2	R^3	Code	R^1	R^2	R^3
BZ_1	Cl	H	CH_2CH_3	BZ_4	Cl	H	$H_2CH_2CH_3$
BZ_2	CH_3	H	CH_2CH_3	BZ_5	H	H	$H_2CH_2CH_3$
BZ_3	CH_3	H	$H_2CH_2CH_3$	BZ_6	H	H	CH_2CH_3

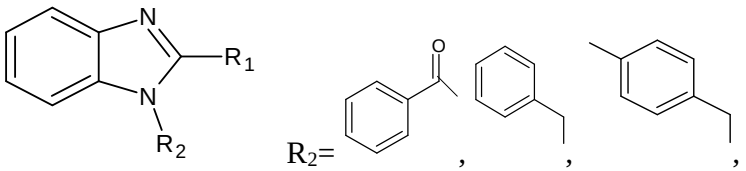
2.8) Sreena K. *et al.*, 2009 had synthesized some benzimidazole derivatives and screened for their anthelmintic activity. The presences of specific functional group were confirmed by IR spectroscopy. The structures for the synthesized compounds were identified by NMR and Mass spectral analysis. Among the synthesized compounds 2-phenyl benzimidazole (Fig-9) showed potent anthelmintic activity.



(Fig-9)

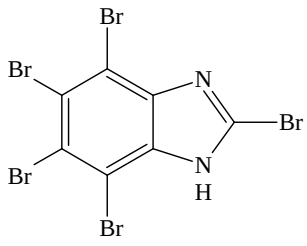
2.9) Podunacvac-Kuzmanovic *et al*., 2009 synthesized a set of benzimidazole derivatives and they were tested for their inhibitory activities against the gram negative bacterium *Pseudomonas aeruginosa* and minimum inhibitory concentration were determined for all the compounds. Quantitative structure activity relationship (QSAR) analysis was applied

to the synthesized derivatives using a combination of various physicochemical, steric, electronic, and structural molecular descriptors. From the QSAR result, they concluded that the 2-amino and 2-methyl benzimidazole derivatives (Fig-10) are effective *in vitro* against the gram negative bacteria *Pseudomonas aeruginosa*.



(Fig-10) $R_{1 =} NH_{2,} CH_3$

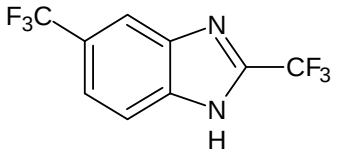
2.10) Alagoz *et al*., 2004 carried out the QSAR studies on 4,5,6,7 tetra-bromo benzimidazole derivatives. The inhibitory activity data (IC_{50}) and the values converted in to –log IC_{50} (μM). From these values confirmed that compound b (Fig-11) had more effective inhibitory concentration.



(Fig-11)

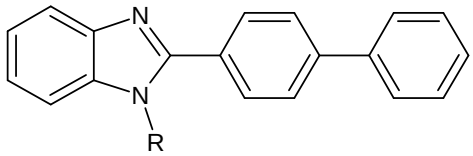
2.11) Bariwal *et al*., 2008 synthesized a series of 2-(triflouromethyl)-1*H*-benzimidazole derivatives with 5th and 6th positions having bio isosteric substituent (-Cl, -F, -CF_3, -CN). Analogues were tested *in vitro* anti-protozoal activity against the protozoan *Giardia intestinal* and *Trichomonas vaginalis* compared with albendazole and metronidazole. The

below mentioned compound (Fig-12) was more active than albendazole against *T. vulgaris* and also showed moderate anti malarial activity against W_2 and D_6 strains of *Plasmodium falciparum.*



(Fig-12)

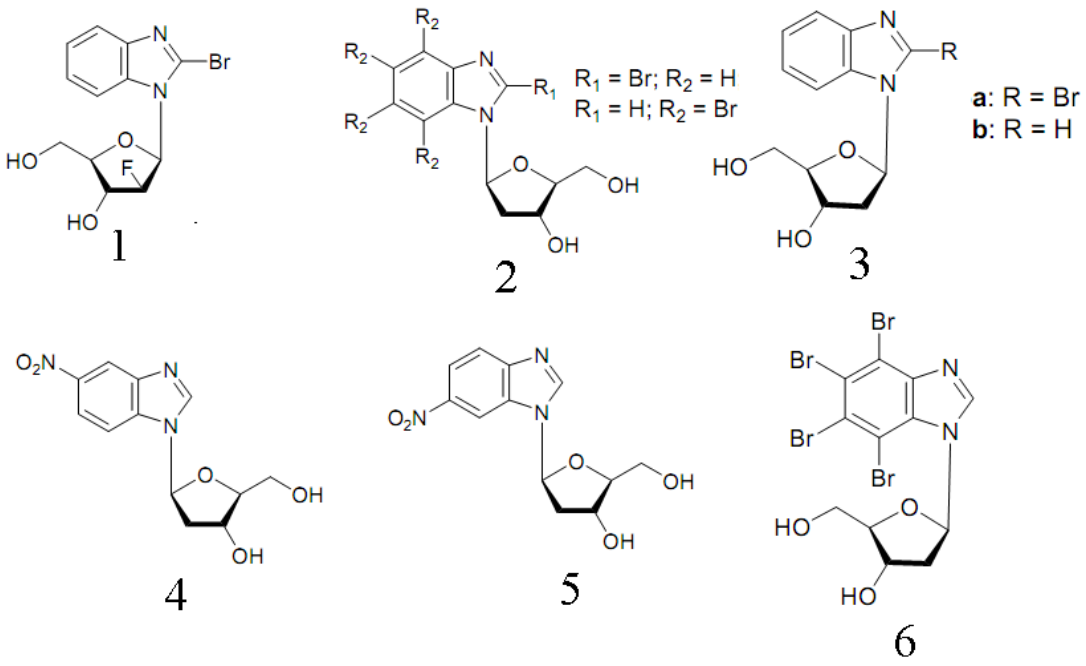
2.12) Alonso *et al.*, 2009 designed a novel and functionalized benzimidazole derivatives. Compounds were tested against PDE-1V for potential anti-asthmatic effect, compound a, b and c shown inhibitory activity (3.40%, 13.52% and 8.91%) at 1μm doses. The compound 'b' (Fig-13) showed potential anti-asthmatic activity.



Compound	R
A	H
B	C_2H_5
C	$CH_2CH_2CH_3$

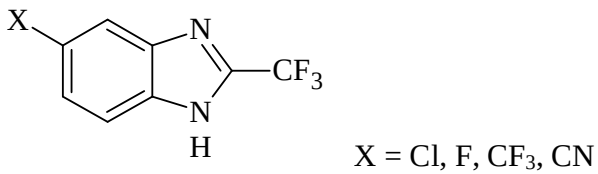
(Fig-13)

2.13) Simone Budow *et al.*, 2009 synthesized a series of benzimidazole derivatives and the β-L- and β-D-2-deoxyribonucleosides substituted benzimidazole tested for anti-viral activity against selected RNA and DNA viruses including HIV-1, BVDV, YFV, DENV-2, WNV, HBV, HCV and human RSV virus. The compounds 1 to 6 (Fig-14) had exhibit good antiviral activities.



(Fig-14)

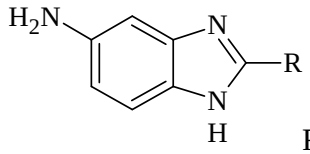
2.14) Andrew *et al.*, 1997 developed a series of 2-(Triflurомethyl)-1H-benzimidazole derivatives (Fig-15) with various 5th and 6th position bio-isosteric substituent's (-Cl, -F, -CF_3, -CN) by using a short synthetic route. Each analogue was tested *in vitro* against the protozoa *Giardia intestinalis* and *Trichomonas vaginalis* in comparison with albendazole and metronidazole.



(Fig-15)

2.15) Mohd Rashid *et al.*, 2011 synthesized 5-amino 2-substituted benzimidazole derivatives (Fig-16) in the presence of formalin. The synthesized compounds were tested

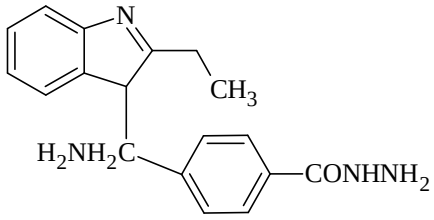
for anti-bacterial activity. All of the compounds showed good anti-bacterial against the growth of *Staph. aureus* and *E. coli*. The amino group at 5^{th} position was important for exhibiting inhibitory activity.



R = ClC_6H_4, OHC_6H_4, $Cl_2C_6H_3$

(Fig-16)

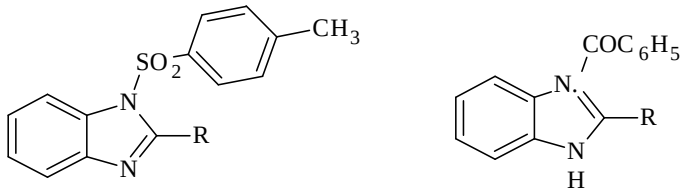
2.16) Masry *et al.*, 2000 discovered a series of 2-alkyl 1-(4'–benzhydrazide) amino methyl benzimidazole derivatives. In which hydrazide derivatives were showed potent inhibitory activity towards the enzyme MAO. The newly synthesized benzimidazole hydrazides were exhibited Low anti-convulsant activity which was found to be maximum with compound R = C_2H_5 (Fig-17).



(Fig-17)

2.17) Gupta S.K. *et al.,* 2010 synthesized 2-alkyl benzimidazole and 2-aryl benzimidazole derivatives by using different acids namely acetic acid, o-chloro benzoic acid, benzoic acid and cinnamic acid. These were further treated with tosyl chloride and benzoyl chloride to get N-substituted benzimidazole derivatives. Final derivatives were tested for anti-microbial activity against *Escherichia coli, Pseudomonas aeruginosa* and *staphylococcus aureus*. Compound 2d and 2d (Fig-18) showed very potent activity

aganist *Pseudomonas aeruginosa*. While 3a and 3d (Fig-18) exhibited average activity against the same organism.

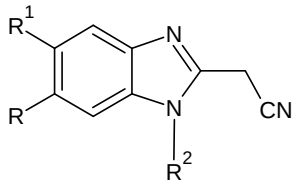


(Fig-18)

2a R = CH_3 3a R = CH_3

2d R = C_8H_7 3d R = C_8H_7

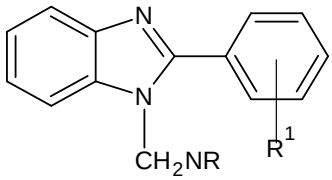
2.18) Raghvendra Dubey *et al.,* 2007 introduced a new method for synthesis of benzimidazole derivatives. It involves condensation of substituted ortho phenylene diamine with cyanoacetic acid in refluxing ethylene glycol. All of them gave excellent yields. Among the all compounds 2a, 2b, 2g (Fig-19) gave more yields.



(Fig-19)

Compound	R	R_1	R_2
2a	H	H	H
2b	CH_3	H	H
2g	H	H	CH_3

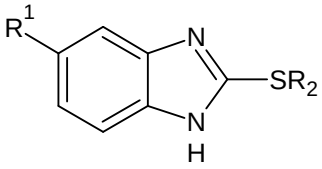
2.19) Leonard *et al.,* 2007 a new series of substituted benzimidazole derivatives and they were characterized by IR, NMR and Mass spectral analysis. The synthesized compounds were evaluated for anti-inflammatory and anti-bacterial activity. All the compounds (Fig-20) exhibited significant to moderate anti-inflammatory and anti-bacterial activities.



(Fig-20)

NR	R^1
Morpholine	3-NO_2
Piperazine	2-NH_2
Piperidine	3-NO_2
Imidazole	3-NO_2
Diphenyl amine	3-NO_2
Piperazine	3-NO_2
Piperidine	2-NH_2

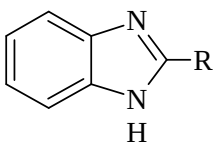
2.20) Kazimierczuk *et al.,* 2005 synthesis 2-polyfluroalkyl and 2-nitrobenzyl sufanyl benzimidazoles. Compounds were evaluated for their activity against mycobacterium strains and compound a and b(Fig-21) showed their MIC values 2 μmol L^{-1} and 4 μmol L^{-1}.



(Fig-21)

Compound	R^1	R^2
A	Cl	Br
B	$C_7H_6NO_2$	C_4F_9

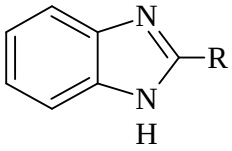
2.21) Niknam K. *et al.,* 2007 adopted a microwave-assisted method for the synthesis of 2-substituted benzimidazoles (Fig-22) in the presence of Alumina-Methanesulfonic acid (AMA). In addition, some new bis-benzimidazoles prepared from the direct reaction of phenylenediamine with di-carboxylic acid under microwave irradiation. This method proved to be advantageous over conventional method with respect to that of reaction time and yield.



(Fig-22)

R= 2-C_7H_7O	R= C_6H_5
R= 2-$C_6H_4O_2$	R= C_6H_4N
R= 2-C_6H_5O	R= C_6H_6N
R= 2-C_6H_4Cl	R= 4-C_6H_4Br

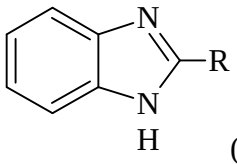
2.22) Na Zhao *et al.,* 2005 synthesized a benzimidazoles (Fig-23) under microwave irradiation by condensing 1, 2 phenylene diamine with different carboxylic acids without catalyst.



(Fig-23)

Compound	R
1	$C_6H_5OCH_2$
2	$2,4(Cl)_2C_6H_3OCH_2$
3	CH_3
4	C_6H_5
5	CH_3
6	$C_{10}H_7CH_2$

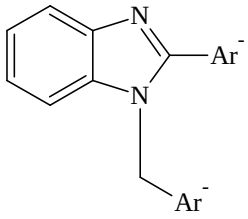
2.23) Rishipathak D.D. *et al.,* 2007 synthesized various 2-alkyl and 2-aryl substituted benzimidazole derivatives (Fig-24) under microwave irradiation. Poly phosphoric acid used as a solvent in this method. The synthesized compounds were identified by IR, NMR, Mass spectral analysis. This method proved to be advantageous over conventional method with respect to reaction time and yield.



(Fig-24)

Compound	R	Compound	R
A	2- CH_3 (Acetic Acid)	B	2- $(CH_2C_6H_5)$
C	2- CH_2Cl	D	2- $CH_2CH_2CH_2CH_2CH_3$
E	C_6H_5 (Benzamide)	F	2- CH_3 (Acetamide)
G	2-(ClC_6H_4)	H	2- C_6H_5 (Benzoic acid)
I	2-m-Tolyl	J	2-$(NH_2C_6H_4)$

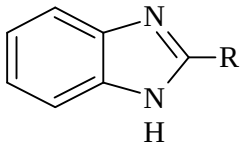
2.24) Perumal S. *et al.,* 2004 synthesized 2-Aryl-arylmethyl-1H-1, 3-benzimidazoles (Fig-25) in the presence of montmorillonite K-10 under Microwave irradiation in the absence of solvent. This synthetic protocol was advantageous over the previous methods as the reaction performed with an environmentally begin clay catalyst, it provides a good yield of product and reaction occurs more rapidly.



Compound	R	Compound	R
A	C_6H_5	F	o-ClC_6H_4
B	p-$MEOC_6H_4$	G	2-Furyl
C	p-MEC_6H_4	H	p-$NO_2C_6H_4$
D	p-ClC_6H_4	I	o-$NO_2C_6H_4$
E	o-$MEOC_6H_4$	J	p-$(CH_3)_2NC_6H_4$

(Fig-25)

2.25) Raghvendra Dubey *et al.,* 2007 synthesized 2-alkyl and 2-Aryl substituted benzimidazole derivatives (Fig-26) by both conventional and microwave method in the presence of polyphosporicacid. They compared the physical properties of derivatives synthesized by both conventional and microwave method and also studied the effect of salt form of reactant for completion of the reaction. The microwave method is more beneficial, in respect of yield and time than conventional method of synthesis.



Code	R
1	H
2	CH_3
3	C_6H_6N
4	C_6H_4Cl

(Fig-26)

Aim And plAn of the work

3. AIM AND PLAN OF WORK

Aim:

Continuous increase in bacterial resistance to existing drugs leading to development of new drugs with antimicrobial activity against drug resistance microorganisms.

From the literature survey, the benzimidazole derivatives have already been assessed for these features of position 4, 6 and 7; however, little work has been directed towards the 5- position of benzimidazole. Introduction of a small substituent in to the 2- and 5- position is characteristic for benzimidazole based anti-helmentics; alternatively, a bulky 2-substitution characterizing drugs used in the treatment of peptic ulcer and are sometimes referred as proton pump inhibitors; bulky 1- and 2-substituents are found in H_1-anti-histaminics. It is suggested that one of the requirement for optimal anti-protozoal action, is that the substituted benzimidazoles must bear a hydrogen atom at the 1-position of benzimidazole ring. *(Goel.P.K. et al. 2007)*

Microwave assisted organic synthesis (MAOS) is an acknowledged quick alternative and green technology in synthetic organic chemistry, and also it is superior in many ways to traditional heating. It can be termed as e-chemistry because it is easy, effective, economic and eco-friendly and is believed to be a step towards green chemistry. Many organic reaction proceeds much faster and with higher yields under microwave irradiation compared to conventional heating.

So, in the present communication, we decided to synthesize 2-substituted benzimidazole derivatives by both conventional and microwave method and compare the yield. Further the study will extended to introduce substitution on 5^{th} position, and to screen the newly synthesized compounds for their anti-bacterial and anti-fungal activity.

PLAN OF THE WORK:

- Synthesis of 2-subsituted benzimidazole derivatives by conventional Method.
- Synthesis of 2-subsituted benzimidazole derivatives by Microwave Method.
- Synthesis of 5-nitro 2-subsituted benzimidazole derivatives.
- Synthesis of 5-amino 2-subsituted benzimidazole derivatives.
- Determination of physical properties of 5-amino 2-subsituted benzimidazole derivatives.
- Spectral characterization of 5-amino 2-subsituted benzimidazole derivatives
- Evaluation of anti-bacterial and anti-fungal activity of 5-amino-2-subsituted benzimidazole derivatives.

EXPERIMENTAL MATERIALS AND INSTRUMENTS

4. EXPERIMENTAL

4.1. Materials and Instruments:

Melting points were determined in open capillary tubes on melting point apparatus (sunbim, Guna enterprises) and are uncorrected. Spectral analyses were performed in the Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology, Madras, using ^{1}H NMR (Bruker-NMR 500 mHZ) spectrometer and Mass (JEOL GC mate) spectrometer. FT-IR (Perkin-Elmer) was performed in Ideal Analytical Research Institute Puduchery, and UV spectra were recorded by using Double beam UV Spectrometer SHIMADZU 1700 at Adhiparasakthi College of Pharmacy, Melmaruvathur.

In ^{1}H NMR, chemical shifts were reported in δ values MeOD and DMSO – *d6* as a solvent and tetramethylsilane as internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, m-multiplet.) in the solvent indicated and IR spectra was recorded in KBr pellets.

All the chemicals and reagents and were commercially available (Rankem, SD fine, Loba and Fluka) and of synthetic grade. Glasswares were as oven or flame dried for moisture sensitive reactions. When necessary, solvents and reagents were dried prior to use. Solution or extracts in organic solvents were dried over anhydrous sodium sulphate or fused calcium chloride before evaporation to under vacuum using rotary evaporator. Analytical samples were dried in vaccum and were free of significant impurities on TLC.

Two gram negative bacterial organisms (*Proteus vulgaris* NCTC 4635, *Klesibella pneumonia* ATCC 29655) and gram positive bacterial organisms (*Bacillus cereus* NL98, *Enterococcus faecium* ATCC 29212) and two fungi strains (*Aspergillus niger* and *Aspergillus fumigatus)* were collected from Microbial Resources Division, Kings Institute of Preventive Medicine, Guindy, Chennai.

4.2 Methodology:

Monitoring of Synthetic Reaction Procedures:

Synthetic procedures were employed for synthesis of compounds SY_1 to SY_{15} and the completion of reactions were monitored by Thin Layer Chromatography (TLC). Silica gel G is used as a adsorbent, the plates were activated by heating at 110° C for one hour. Methanol: water, Methanol: Chloroform mixtures (9:1, 8:2, 7:3) were used as a Mobile phase. The plates were visualized by UV light, iodine chamber.

Purification Techniques:

Recrystallization: The crude products were recrystallized by charcoal treatment with appropriate solvent. Single solvent was used wherever possible and solvent mixtures were not used anywhere.

Authentication of Chemical Structures and Purity of Compounds:

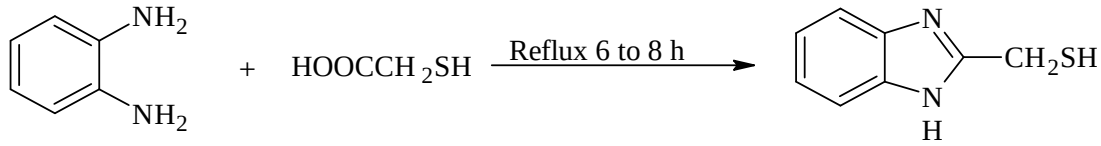
Chemical structure of synthesized compounds and their purity were identified by thin layer chromatography, UV-visible spectrometer, melting point and various spectral techniques including Fourier Transform Infra Red Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Mass Spectroscopy and Ultra Violet Spectrophotometry.

4.3. Synthesis of compounds:

4.3.1. Synthesis of 1 *H*-benzimidazol-2-yl methane thiol: (SY1)

a. Conventional method:

O-phenylene diamine 27 g (0.25 M) and thioglycollic acid 31.28 g (0.34 M) was heated on a water bath at 100° C for 6-8 h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using of 10% sodium hydroxide solution. The crude benzimidazole was filtered at the pump, washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added, digested for 15 min. The solution was filtered while hot, cooled the filtrate to about 10° C. The pure product was filtered, washed with 25 ml of cold water and dried at 100° C. (*Vogel's 2006, Ahuluwalia et al., 2000*)



O-phenylene diamine thioglycollic acid 1 H-benzimidazol-2-yl methane thiol

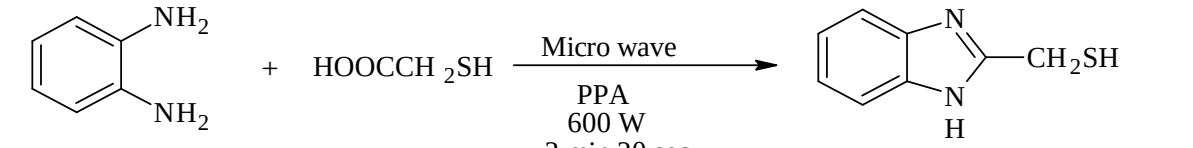
Scheme- 1.a: Synthesis of 1 *H*-benzimidazol-2-yl methane thiol

b. Microwave method:

O-phenylene diamine 1.08 g (0.01 M), thioglycollic acid 0.92 g (0.01 M) and poly phosphoric acid 10 g was properly mixed with glass rod in a beaker. The mixture was

irradiated in the microwave oven for 2 min 20 sec at 600 W. The completion of reaction was monitored by TLC, after irradiation the mixture was poured into ice cold water and then slowly neutralized with sodium hydroxide to pH 8. The precipitate was collected by filtration, dried and recrystallized from hot water.

(*Rishipathak et al., 2007, Perumal et al., 2004*)



O-phenylene diamine thioglycollic acid 1 H-benzimidazol-2-yl methane thiol

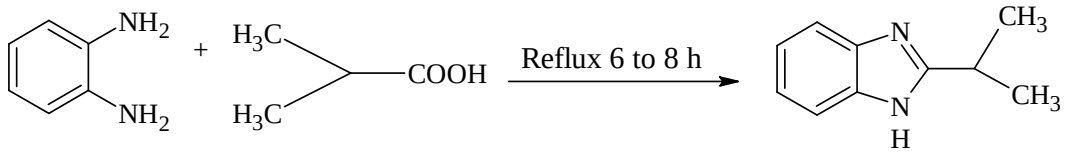
Scheme- 1.b: Synthesis of 1 *H*-benzimidazol-2-yl methane thiol

4.3.2. Synthesis of 2-(propan-2-yl)-1*H*-benzimidazole: (SY2)

a. Conventional method:

O-phenylene diamine 27 g (0.25 M) and isobutyric acid 29.92 g (0.34 M) was heated on a water bath at 100^{o} C for 6-8 h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using of 10% sodium hydroxide solution. The crude benzimidazole was filtered at the pump, washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added, digested for 15 min. The solution was filtered while hot, cooled the filtrate to about 10^{o} C. The pure product

was filtered, washed with 25 ml of cold water and dried at 100^{o} C. (*Vogel's 2006, Ahuluwalia et al., 2000*)



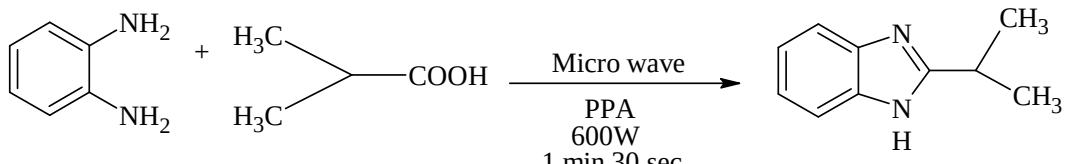
O-phenylene diamine isobutyric acid 2-(propan-2-yl)-1*H*-benzimidazole

Scheme- 2.a: Synthesis of 2-(propan-2-yl)-1*H*-benzimidazole

b. Microwave method:

O-phenylene diamine 1.08 g (0.01 M), isobutyric acid 0.88 g (0.01 M) and poly phosphoric acid 10 g was properly mixed with glass rod in a beaker. The mixture was irradiated in the microwave oven for 1 min 30 sec at 600 W. The completion of reaction was monitored by TLC, after irradiation the mixture was poured into ice cold water and then slowly neutralized with sodium hydroxide to pH 8. The precipitate was collected by filtration, dried and recrystallized from hot water.

(*Rishipathak et al., 2007, Perumal et al., 2004*)



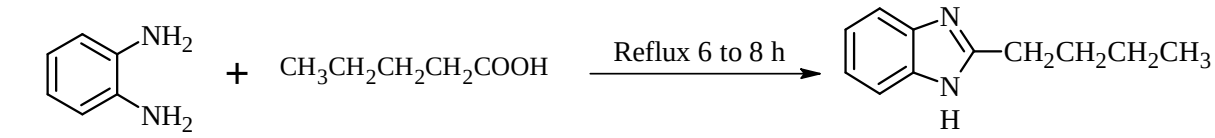
O-phenylene diamine isobutyric acid 2-(propan-2-yl)-1*H*-benzimidazole

Scheme- 2.b: Synthesis of 2-(propan-2-yl)-1*H*-benzimidazole

4.3.3. Synthesis of 2-butyl-1*H*-benzimidazole: (SY3)

a. Conventional method:

O-phenylene diamine 27 g (0.25 M) and valeric acid 34.68 g (0.34 M) was heated on a water bath at 100^{o} C for 6-8 h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using of 10% sodium hydroxide solution. The crude benzimidazole was filtered at the pump, washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added, digested for 15 min. The solution was filtered while hot, cooled the filtrate to about 10^{o} C. The pure product was filtered, washed with 25 ml of cold water and dried at 100^{o} C. (*Vogel's 2006, Ahuluwalia et al., 2000*)



O-phenylene diamine valeric acid 2-butyl-1*H*-benzimidazole

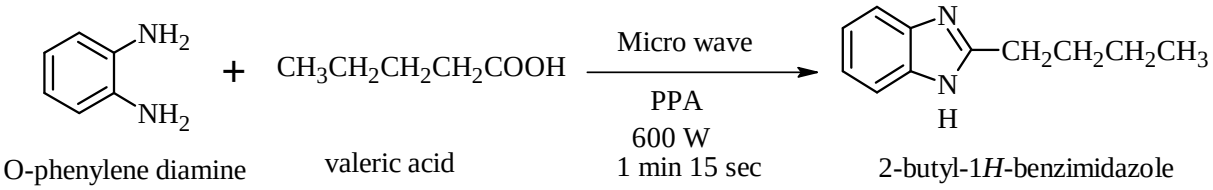
Scheme- 3.a: Synthesis of 2-butyl-1*H*-benzimidazole

b. Microwave method:

O-phenylene diamine 1.08 g (0.01 M), valeric acid 1.02 g (0.01 M) and poly phosphoric acid 10 g was properly mixed with glass rod in a beaker. The mixture was irradiated in the microwave oven for 1 min 15 sec at 600 W. The completion of reaction was monitored by TLC, after irradiation the mixture was poured into ice cold water and

then slowly neutralized with sodium hydroxide to pH 8. The precipitate was collected by filtration, dried and recrystallized from hot water.

(*Rishipathak et al., 2007, Perumal et al., 2004*)



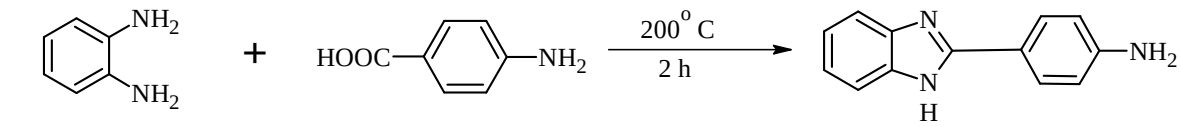
O-phenylene diamine — valeric acid — 1 min 15 sec — 2-butyl-1*H*-benzimidazole

Scheme- 3.a: Synthesis of 2-butyl-1*H*-benzimidazole

4.3.4. Synthesis of (1*H*-benzimidazol-2-yl) aniline: (SY4)

a. Conventional method:

A mixture of O-phenylene diamine 3.8 g (34 mM) and 4-amino benzoic acid 4.5 g (33 mM) were stirred in a syrupy O-phosphoric acid (45 ml) at 200º C for 2 h. The reaction mixture was cooled and poured on to the crushed ice. The bulky white precipitate obtained was stirred in cold water (400 ml) and sodium hydroxide solution (5 M) was added until the pH 7. The resulting solid was filtered and recrystallized from methanol. (*Sreena et al., 2009*)



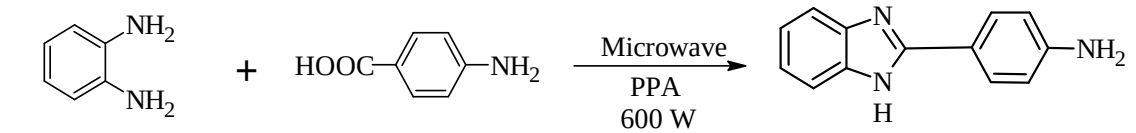
O-phenylene diamine — 4-amino benzoic acid — 4-(1*H*-benzimidazol-2-yl) aniline

Scheme- 4.a: Synthesis of (1*H*-benzimidazol-2-yl) aniline

b. Microwave method:

O-phenylene diamine 1.08 g (0.01 M), 4-amino benzoic acid 1.37 g (0.01 M) and poly phosphoric acid 10 g was properly mixed with glass rod in a beaker. The mixture was irradiated in the microwave oven for 6 min 30 sec at 600 W. The completion of reaction was monitored by TLC, after irradiation the mixture was poured into ice cold water and then slowly neutralized with sodium hydroxide to pH 8. The precipitate was collected by filtration, dried and recrystallized from methanol.

(*Rishipathak et al., 2007, Perumal et al., 2004*)



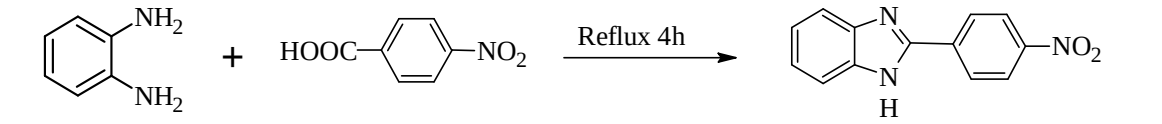
O-phenylene diamine 4-aminobenzoic acid 4-(1*H*-benzimidazol-2-yl)aniline

Scheme- 4.b: Synthesis of (1*H*-benzimidazol-2-yl) aniline

4.3.5. Synthesis of 2-(4-nitrophenyl)-1*H*-benzimidazole: (SY5)

a. Conventional method:

O-phenylene diamine 1.08 g (0.01 M) and 4-nitro benzoic acid 1.69 g (0.01 M) in 20 ml acetic acid was refluxed for 4 h. The precipitate obtained after cooling was recrystallized from ethanol. (*Mohamed al messmary et al., 2010*)



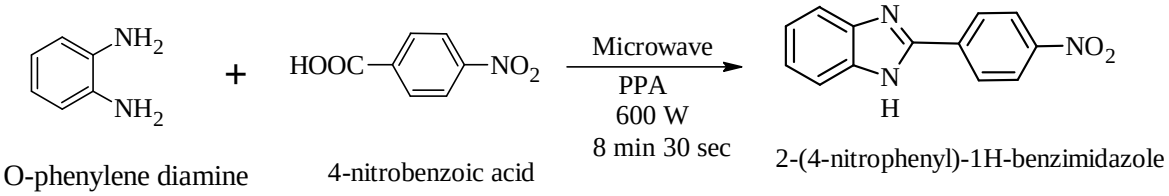
O-phenylene diamine 4-nitro benzoic acid 2-(4-nitrophenyl)-1H-benzimidazole

Scheme- 5.a: Synthesis of 2-(4-nitrophenyl)-1*H*-benzimidazole

b. Microwave method:

O-phenylene diamine 1.08 g (0.01 M), 4-nitro benzoic acid 1.69 g (0.01 M) and poly phosphoric acid 10 g was properly mixed with glass rod in a beaker. The mixture was irradiated in the microwave oven for 8 min 30 sec at 600 W. The completion of reaction was monitored by TLC, after irradiation the mixture was poured into ice cold water and then slowly neutralized with sodium hydroxide to pH 8. The precipitate was collected by filtration, dried and recrystallized from ethanol.

(*Rishipathak et al., 2007, Perumal et al., 2004*)

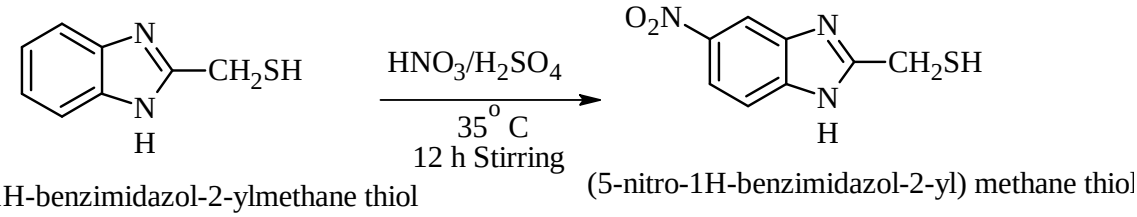


Scheme- 5.b: Synthesis of 2-(4-nitrophenyl)-1*H*-benzimidazole

4.3.6. Synthesis of (5-nitro-1*H*-benzimidazol-2-yl) methane thiol: (SY6)

Conc. HNO_3 (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly Conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, 1H-benzimidazol-2yl-methane thiol 4.59 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous

stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)

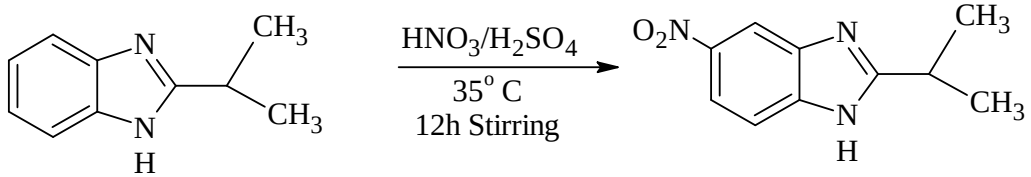


1H-benzimidazol-2-ylmethane thiol (5-nitro-1H-benzimidazol-2-yl) methane thiol

Scheme- 6: Synthesis of (5-nitro-1*H*-benzimidazol-2-yl) methane thiol

4.3.7. Synthesis of 5-nitro 2-(propan-2-yl)-1*H*-benzimidazole: (SY7)

Conc. HNO_3 (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly Conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, 2-(propan-2-yl)-1*H*-benzimidazole 4.48 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)

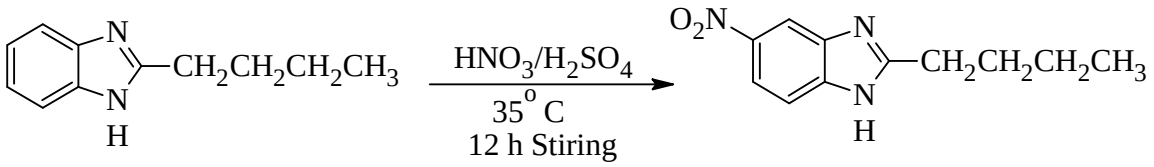


2-(propan-2-yl)-1H-benzimidazole 5-nitro-2-(propan-2-yl)-1H-benzimidazole

Scheme- 7: Synthesis of 5-nitro 2-(propan-2-yl)-1*H*-benzimidazole

4.3.8. Synthesis of 5-nitro 2-butyl-1*H*-benzimidazole: (SY8)

Conc. HNO_3 (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly Conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, 2-butyl-1*H*-benzimidazole 4.87 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)

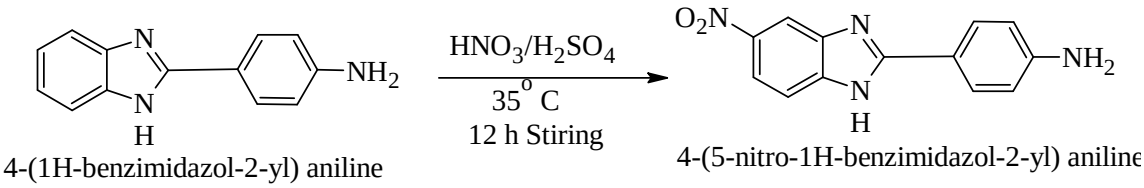


2-butyl-1H-benzimidazole 5-nitro 2-butyl-1H-benzimidazol

Scheme- 8: Synthesis of 5-nitro 2-butyl-1*H*-benzimidazole

4.3.9. Synthesis of 4-(5-nitro-1*H*-benzimidazol-2-yl) aniline: (SY9)

Conc. HNO_3 (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly Conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, (1*H*-benzimidazol-2-yl) aniline 5.85 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)



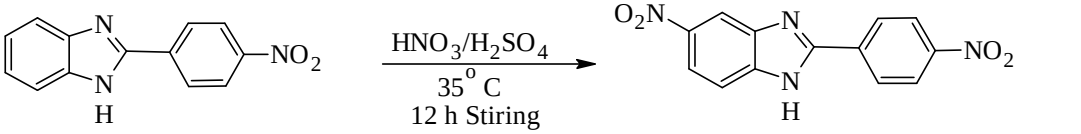
4-(1H-benzimidazol-2-yl) aniline

4-(5-nitro-1H-benzimidazol-2-yl) aniline

Scheme- 9: Synthesis of4-(5-nitro-1*H*-benzimidazol-2-yl) aniline

4.3.10. Synthesis of 5-nitro 2-(4-nitrophenyl)-1*H*-benzimidazole: (SY10)

Conc. HNO_3 (7.5 ml) was placed in 3-necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly Conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, 2-(4-nitrophenyl)-1*H*-benzimidazole 6.69 g (0.028 M) was added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35º C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol. (*Jitender Singh et al., 2010*)



2-(4-nitrophenyl)-1H-benzimidazole

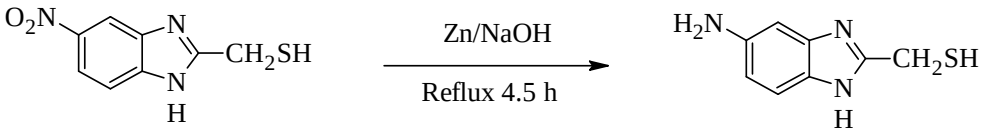
5-nitro 2-(4-nitrophenyl)-1H-benzimidazole

Scheme- 10: Synthesis of 5-nitro 2-(4-nitrophenyl)-1*H*-benzimidazole

4.3.11. Synthesis of (5-amino-1*H*-benzimidazol-2-yl) methane thiol: (SY11)

A solution of 0.5 g of (5-nitro-1*H*-benzimidazol-2-yl) methane thiol in 15 ml of rectified spirit was taken in round bottom flask. To this, 5 ml of 20 % sodium hydroxide and 2.5 g of zinc dust powder was added. The reaction mixture was refluxed until colour

of the solution changed from deep red to colourless (about 4.5 h), the hot mixture was filtered. The zinc residue was return to the flask and extracted with 10 ml of hot rectified sprit for two times. The extracts were combined; the solvent was removed under vacuum, yielded the brown solid and recrystallized from methanol. (*Raju et al., 2009*)

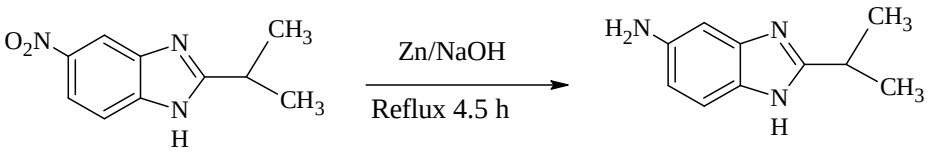


(5-nitro-1H-benzimidazol-2-yl)methanethiol (5-amino-1H-benzimidazol-2-yl)methanethiol

Scheme- 11: Synthesis of (5-amino-1*H*-benzimidazol-2-yl) methanethiol

4.3.12. Synthesis of 5-amino 2-(propan-2-yl)-1*H*-benzimidazole: (SY12)

A solution of 0.5 g of 5-nitro-2-(propan-2-yl)-1*H*-benzimidazole in 15 ml of rectified spirit was taken in round bottom flask. To this, 5 ml of 20 % sodium hydroxide and 2.5 g of zinc dust powder was added. The reaction mixture was refluxed until colour of the solution changed from deep red to colourless (about 4.5 h), the hot mixture was filtered. The zinc residue was return to the flask and extracted with 10 ml of hot rectified sprit for two times. The extracts were combined; the solvent was removed under vacuum, yielded the brown solid and recrystallized from methanol. (*Raju et al., 2009*)

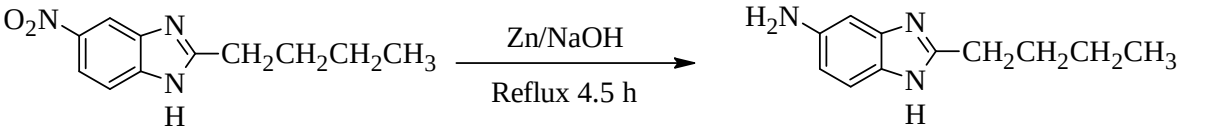


5-nitro-2-(propan-2-yl)-1H-benzimidazole 5-amino 2-(propan-2-yl)-1H-benzimidazole

Scheme- 12: Synthesis of 5-amino 2-(propan-2-yl)-1*H*-benzimidazole

4.3.13. Synthesis of 5-amino 2-butyl-1*H*-benzimidazole: (SY13)

A solution of 0.5 g of 5-nitro 2-butyl-1*H*-benzimidazole in 15 ml of rectified spirit was taken in round bottom flask. To this, 5 ml of 20 % sodium hydroxide and 2.5 g of zinc dust powder was added. The reaction mixture was refluxed until colour of the solution changed from deep red to colourless (about 4.5 h), the hot mixture was filtered. The zinc residue was return to the flask and extracted with 10 ml of hot rectified sprit for two times. The extracts were combined; the solvent was removed under vacuum, yielded the brown solid and recrystallized from methanol. (*Raju et al., 2009*)



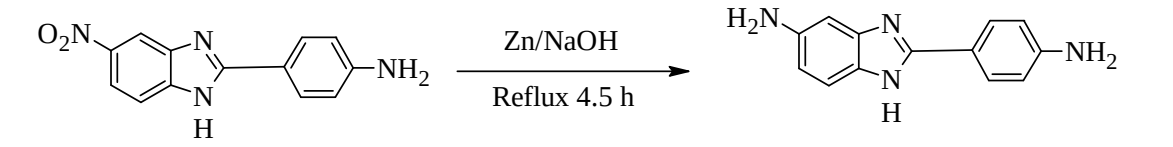
5-nitro 2-butyl-1H-benzimidazole

5-amino 2-butyl-1H-benzimidazole

Scheme- 13: Synthesis of 5-amino 2-butyl-1*H*-benzimidazole

4.3.14. Synthesis of 5-amino 2-(4-aminophenyl)-1*H*-benzimidazole: (SY14)

A solution of 0.5 g of 4-(5-nitro-1*H*-benzimidazol-2-yl) aniline in 15 ml of rectified spirit was taken in round bottom flask. To this, 5 ml of 20 % sodium hydroxide and 2.5 g of zinc dust powder was added. The reaction mixture was refluxed until colour of the solution changed from deep red to colourless (about 4.5 h), the hot mixture was filtered. The zinc residue was return to the flask and extracted with 10 ml of hot rectified sprit for two times. The extracts were combined; the solvent was removed under vacuum, yielded the brown solid and recrystallized from methanol. (*Raju et al., 2009*)



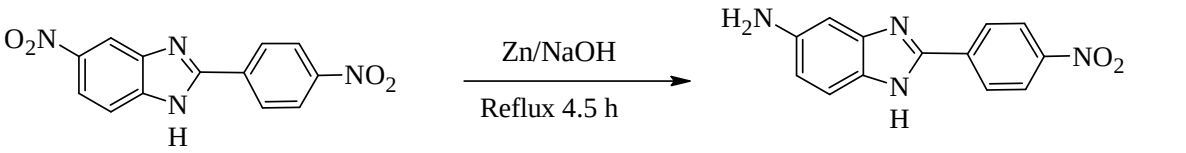
4-(5-nitro-1H-benzimidazol-2-yl)aniline

5-amino 2-(4-aminophenyl)-1H-benzimidazole

Scheme- 14: Synthesis of 5-amino 2-(4-aminophenyl)-1*H*-benzimidazole

4.3.15. Synthesis of 5-amino 2-(4-nitrophenyl)-1*H*-benzimidazole: (SY15)

A solution of 0.5 g of 5-nitro 2-(4-nitrophenyl)-1*H*-benzimidazole in 15 ml of rectified spirit was taken in round bottom flask. To this, 5 ml of 20% sodium hydroxide and 2.5 g of zinc dust powder was added. The reaction mixture was refluxed until colour of the solution changed from deep red to colourless (about 4.5 h), the hot mixture was filtered. The zinc residue was return to the flask and extracted with 10 ml of hot rectified sprit for two times. The extracts were combined; the solvent was removed under vacuum, yielded the brown solid and recrystallized from methanol. (*Raju et al., 2009*)



2-(4-nitrophenyl)-1H-benzimidazole

5-amino 2-(4-nitrophenyl)-1H-benzimidazole

Scheme- 15: Synthesis of 5-amino 2-(4-nitrophenyl)-1*H*-benzimidazole

BIOLOGICAL SCREENING

5. BIOLOGICAL SCREENING

5.1. Evaluation of *in vitro* anti-bacterial activity:

The synthesized compounds (SY_{11}-SY_{15}) were tested for anti-bacterial activity by disc diffusion method. They were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. A final inoculum of 100 µl suspension containing 10^8 CFU/ ml of each bacterium was used. Nutrient agar medium was prepared and sterilized by an autoclave (121° C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with gram negative bacterial organisms *Proteus vulgaris* NCTC 4635, *Klesibella pneumonia* ATCC 29655 and gram positive bacterial organisms *Bacillus cereus* NL98, *Enterococcus faecium* ATCC 29212 in sterile nutrient agar medium at 45° C under aseptic condition. Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25, 100 µg/disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ciprofloxacin (100 µg /disc) was used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 h at 37 ± 1° C. The zone of inhibition (Table-3) was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.

5.2. Evaluation of *in vitro* anti-fungal activity:

The synthesized compounds (SY_{11}-SY_{15}) were tested for anti-fungal activity by disc diffusion method. They were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. A final inoculum of 100 µl suspension containing 10^8 CFU/ ml of each fungus was used. Sabouraud dextrose agar medium was prepared and sterilized by an autoclave (121º C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with fungal organism *Aspergillus niger* and *Aspergillus fumigatus* in sterile sabouraud dextrose agar medium at 45^o C in aseptic condition. Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25, 100 µg/disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ketaconazole (100 µg /disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 48 h at 37±1^o C for antifungal activity. The zone of inhibition (Table-4) was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.

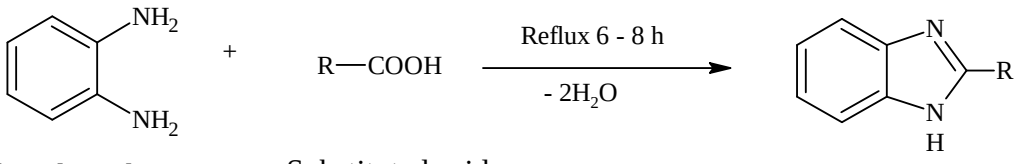
RESULTS AND DISCUSSION

6. RESULTS AND DISCUSSION

6.1. Synthetic Scheme

There are several methods reported for synthesis of 2-substituted benzimidazole derivatives. Mainly two methods are widely employed, which are coupling of O-phenylene diamine with different substituted organic acid in the presence of strong acidic medium (it also done through microwave irradiation) and the coupling of O-phenylene diamine with different substituted aldehydes.

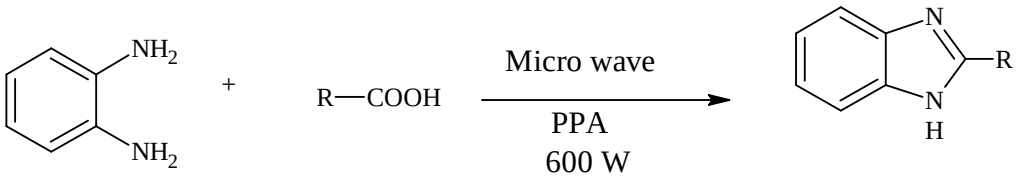
a. Conventional method



O-phenylene diamine Substituted acids 2-Substituted benzimidazoles

(SY 1 - SY 5)

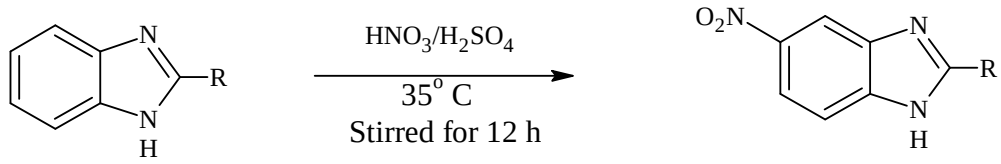
b. Micro wave method



O-phenylene diamine Substituted acids 2-Substituted benzimidazole

(SY 1 - SY 5)

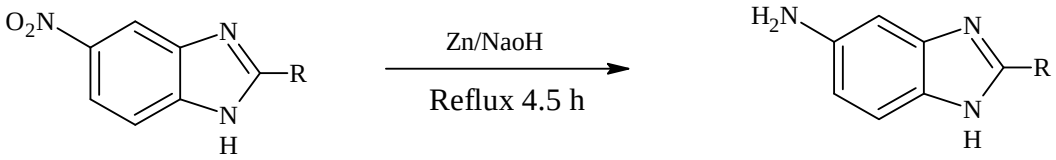
Step 2:-



2-Substituted benzimidazoles

5-Nitro 2-substituted benzimidazole (SY 6-SY 10)

Step 3:-



5-Nitro 2-substituted benzimidazoles

5-Amino 2-substituted benzimidazoles (SY 11-SY 15)

Code	R
SY 1, SY6, SY 11	CH_2SH
SY 2, SY 7, SY 12	$CH(CH_3)_2$
SY 3, SY 8, SY 13	$CH_2CH_2CH_2CH_3$
SY 4, SY 9, SY 14	C_6H_6N
SY 5, SY 10, SY 15	$C_6H_4NO_2$

Scheme-16: General synthetic route for 2, 5 di substituted benzimidazole derivatives

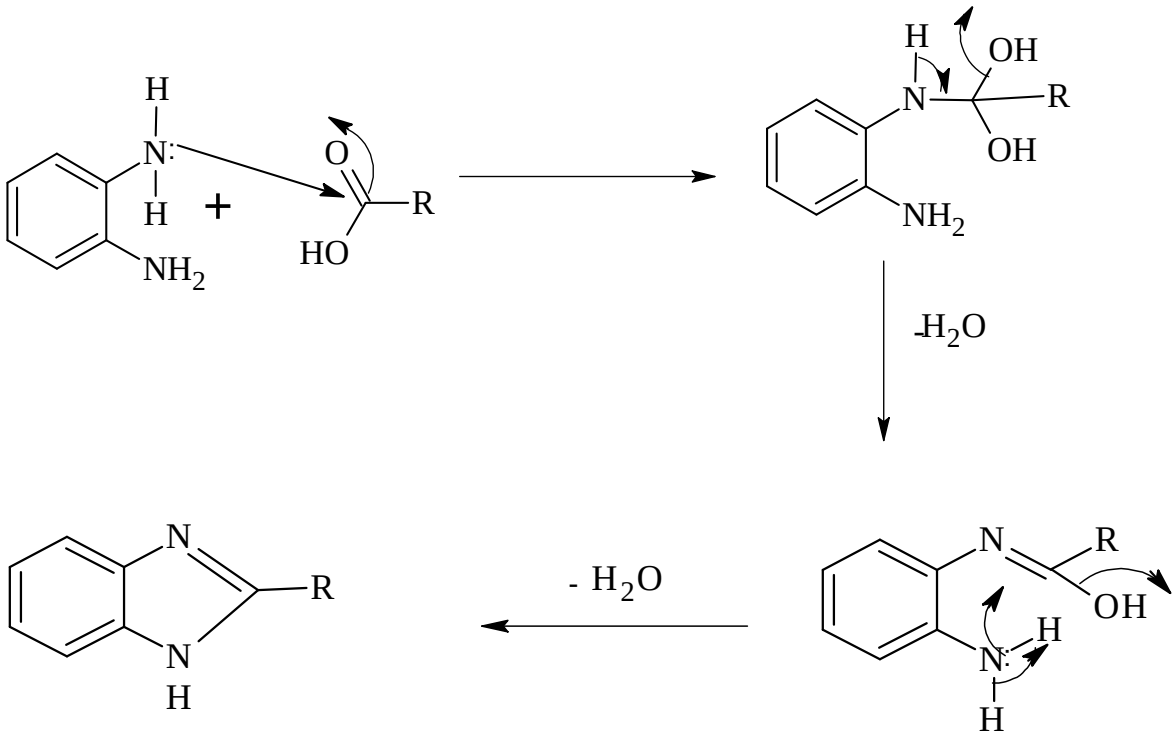
Various oxidative and catalytic reagents such as sulfamic acid, DDQ, air, Oxone, $FeCl_3 \cdot 6H_2O$, In $(OTf)_3$, Yb $(OTf)_3$, 1 Sc $(OTf)_3$, $KHSO_4$, IL, have been employed. Because of the availability of a vast number of aldehydes, the condensation of phenylene

diamines and aldehydes has been extensively used. While many published methods are effective, some of these methods suffer from one or more disadvantages such as high reaction temperature, prolonged reaction time, and toxic solvents etc. Long reaction times for this reaction have been mitigated by the use of microwave heating, both with and without poly phosphoric acid. *(Hanxiangming et al., 2007).*

Compounds in which the aromatic (or) aliphatic ring is directly attached to the second position of the benzimidazole ring were substituted by our scheme. In this method benzimidazole were synthesized from O-phenylene diamine by reaction with mono aliphatic or aromatic carboxylic acids. The reaction was neutralized with sodium hydroxide solution and the crude benzimidazoles (SY_1-SY_5) were isolated by filtration. *(Goel. P. K, et al., 2007).*

SY_1-SY_5 was then subjected to nitration using a mixture (1:1) of Conc. HNO_3 and Conc. H_2SO_4. Earlier studies with benzimidazolinone suggest that it acetic anhydride, nitration product generated depends upon the temperature employed. At 30° C specifically, the 5-nitro derivative was obtained whereas at 70° C, 5, 6-dinitro product was formed. On the basis, we carried out nitration of SY_1-SY_5 under controlled temperature condition at 35° C resulting in the exclusive 5-nitro benzimidazole derivative which was confirmed by spectral analysis.

SY_6-SY_{10} was then subjected to reduction using a mixture of zinc and sodium hydroxide. The resulting product was obtained by heating the reaction mixture up to 4.5 h. Finally the compounds were confirmed by spectral analysis. (*jag mohan, 2006)*



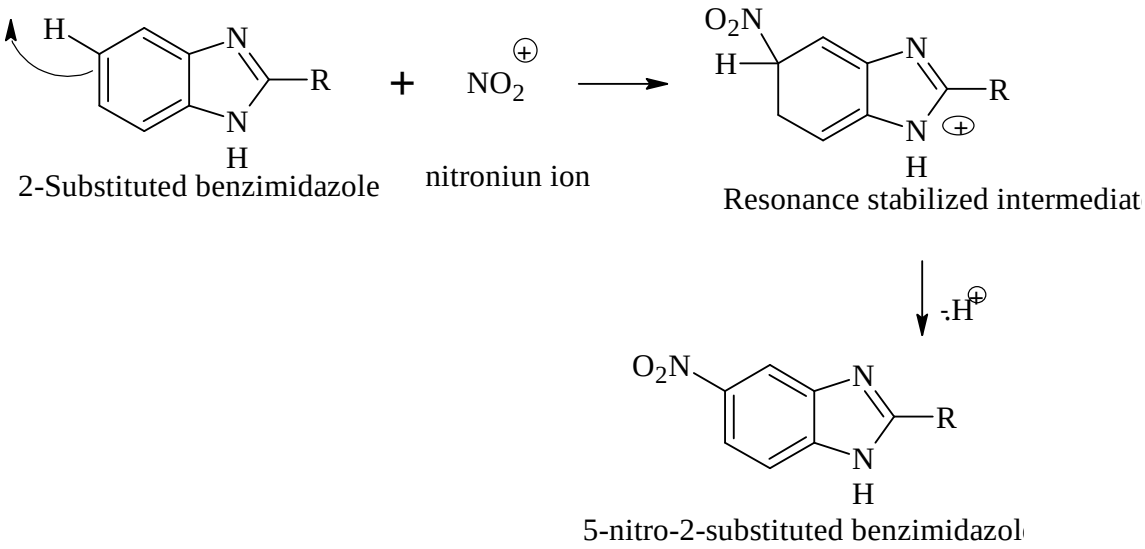
Scheme-17: Mechanism of 2-substituted benzimidazoles

The sulphuric acid convert's nitric acid into highly reactive nitronium ion (NO_2^+) which is an electrophile and attacks the position of highest electron density in the molecule to form a resonance stabilized intermediate which then eliminates a proton to form nitrated derivative. *(Vishnoi N.K, 1996)*

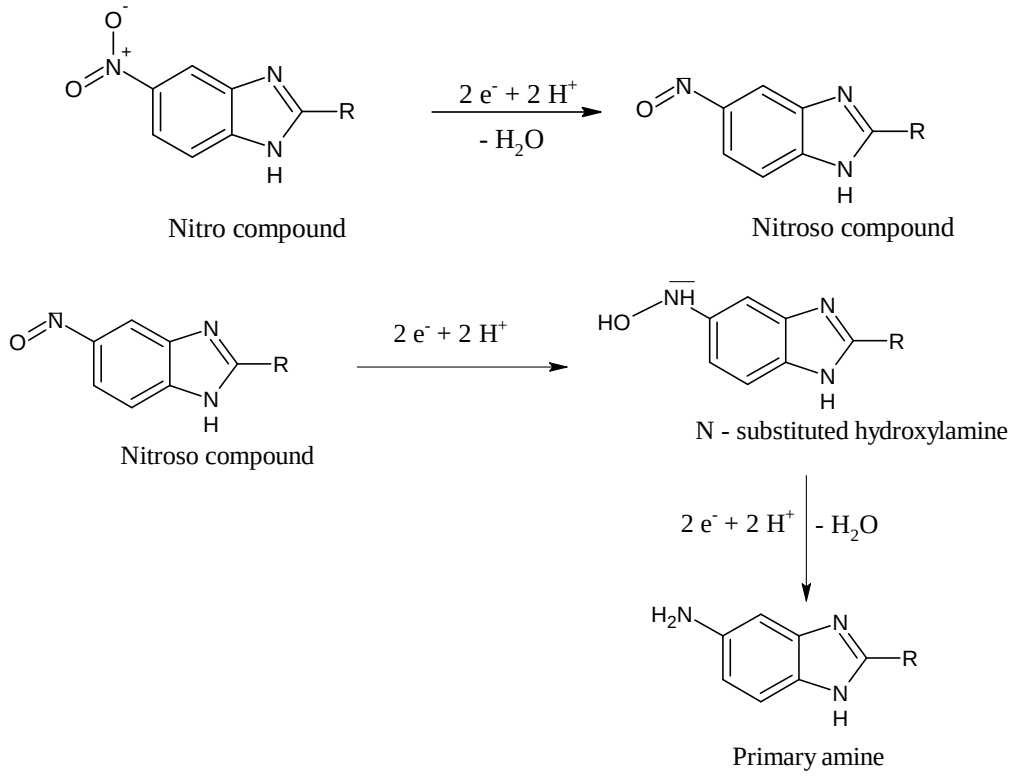
$$H_2SO_4 + HNO_3 \longrightarrow (H_2NO_3)^{\oplus} + HSO_4^{\ominus}$$

$$(H_2NO_3)^{\oplus} \longrightarrow H_2O + NO_2^{\oplus}$$

$$H^+ + HSO_4^- \longrightarrow H_2SO_4$$



Scheme-18: Nitration mechanism of 2-substituted benzimidazole



Scheme-19: Reduction mechanism of 5 nitro 2-substituted benzimidazole

6.2. Interpretation of Spectral data of synthesized compounds (SY_1-SY_{15}):

6.2.1 Spectral analysis of 1 *H*-benzimidazol-2-yl methane thiol

UV: (Fig-27)

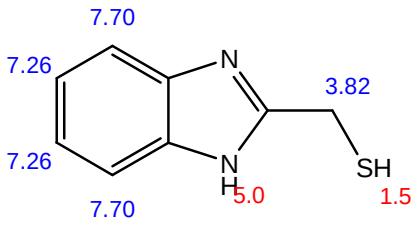
λ_{max} (MeOH)	280.5 **(**ε_{max} 0.9941)
λ_{max} (MeOH)	242.5 **(**ε_{max} 0.8943)

IR (KBr): (Fig-28)

Wave Number (Cm^{-1})	**Assignment**
3369.99	N-H stretching
3100.50	Aromatic C-H stretching
3058.43	Aromatic C-H stretching
2539.91	S-H stretching
1661.54	Aromatic C=C stertching
1556.06	C=N stretching
1484.89	C-H bending(scissoring)
1361.16	C-H bending (wagging)
1271.0	C-N stretching

736.66	C-H bending (rocking)
670.44	C-S stretching

NMR (MeOD): (Fig-29)



(4 aromatic protons, 2 aliphatic protons, 1 proton on nitrogen and 1 proton on sulphur)

δ	**Assignment**
7.70	(2H, m, Ar-H- C_4&C_7)
7.26	(2H, m, Ar-H- C_5&C_6)
5.0	(1H, s, broad, NH)
3.82	(2H, s, CH_2)
1.5	(1H, s, SH)

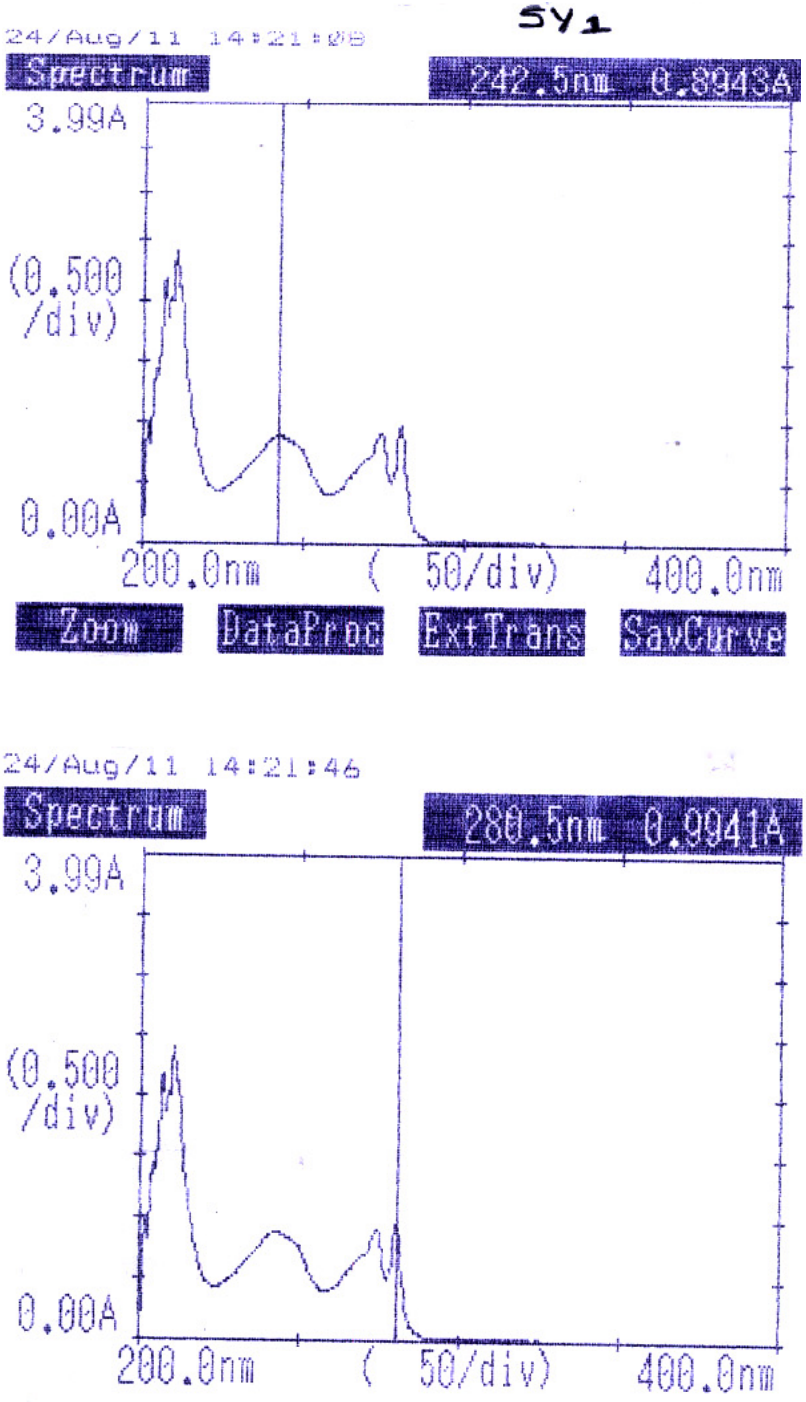


Fig27: UV Spectrum of compound SY_1

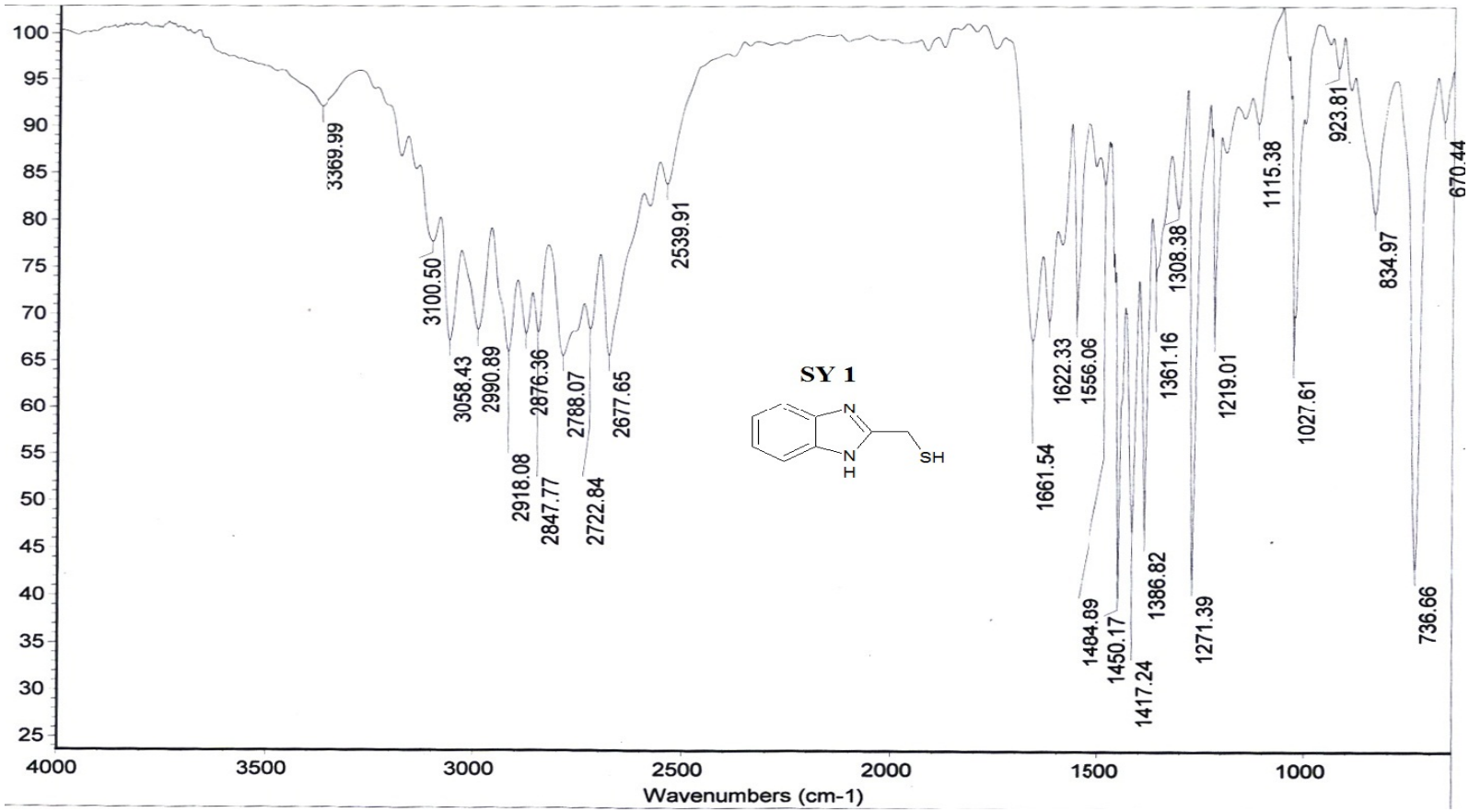


Fig-28: IR Spectrum of compound SY_1

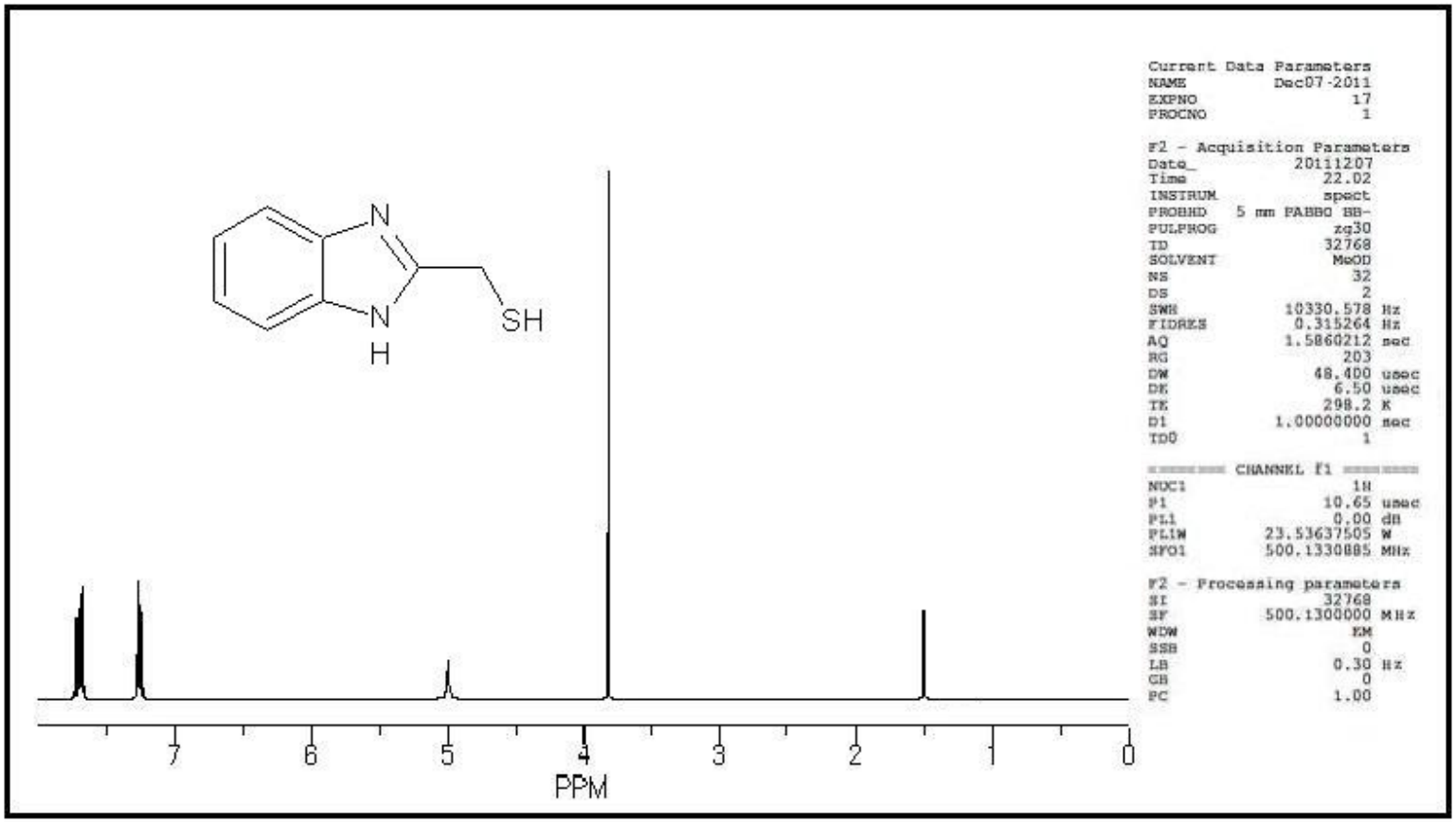


Fig-29: ^{1}H-NMR Spectra of the compound SY_1

6.2.2. Spectral analysis of 2-(propan-2-yl)-1*H*-benzimidazole:

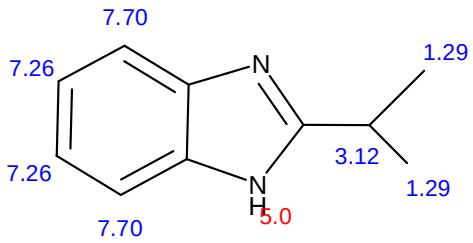
UV: (Fig-30)

λ_{max} (MeOH)	275.5 **(**ε_{max} 0.0287)
λ_{max} (MeOH)	210.0 **((**ε_{max} 1.6715)

IR (KBr): (Fig-31)

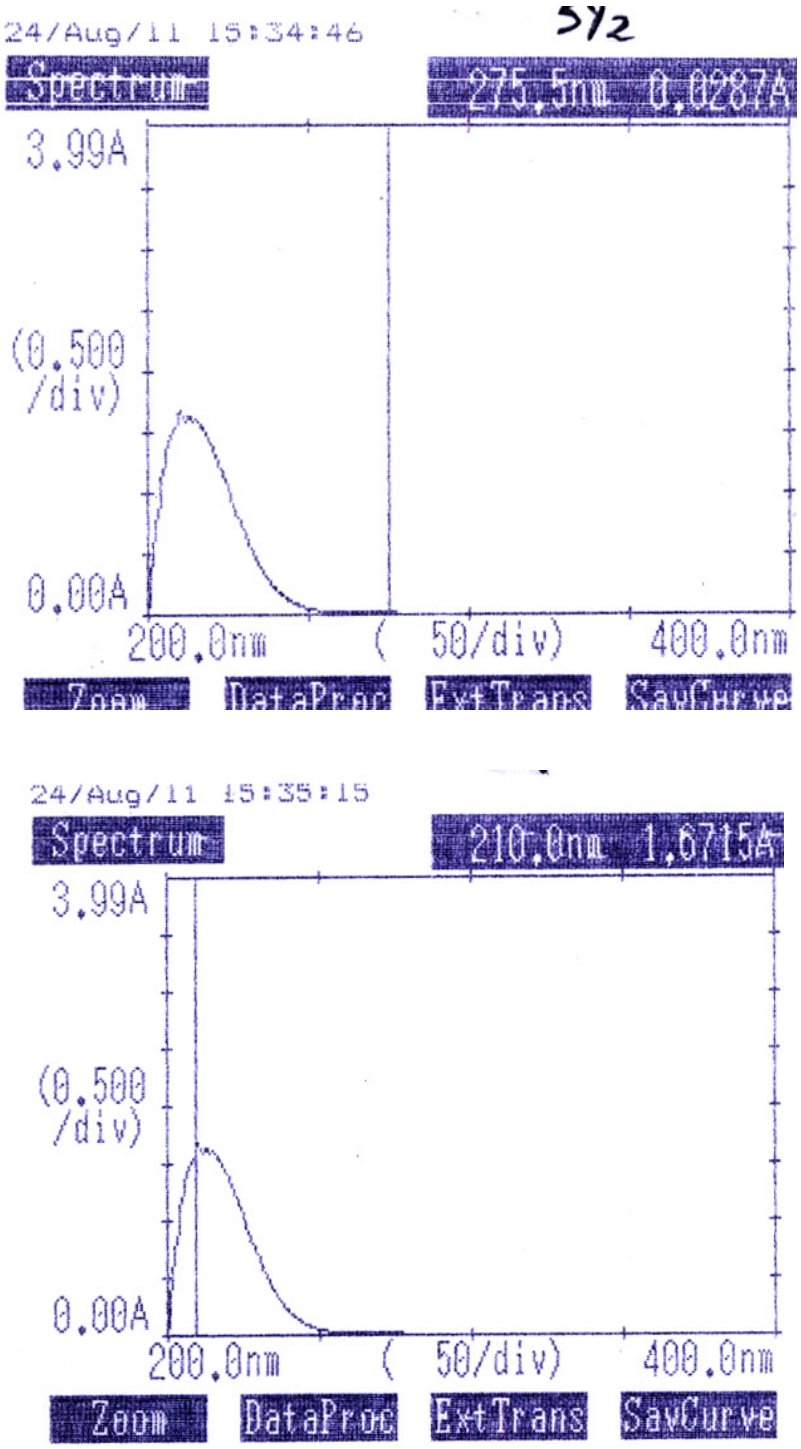
Wave Number (Cm^{-1})	**Assignment**
3363.57	N-H stretching
3032.10	Aromatic C-H stretching
2667.83	C-H stretching
1632.61	C=C stretching
1591.92	C=N stretching
1320.5	C-H bending (gem-dimethyl)
1272.31	C-N stretching
1114.79	C-C stretching

NMR (MeOD): (Fig-32)



(4 aromatic protons, 7 aliphatic protons, and 1 proton on nitrogen)

δ	**Assignment**
7.70	(2H, m, Ar-H- C_4&C_7)
7.26	(2H, m, Ar-H- C_5&C_6)
5.0	(1H, s, broad, NH)
3.12	(1H, m, CH of iso propyl)
1.29	(6H, d, 2CH_3 of iso propyl)



(Fig-30: UV Spectrum of compound SY_2)

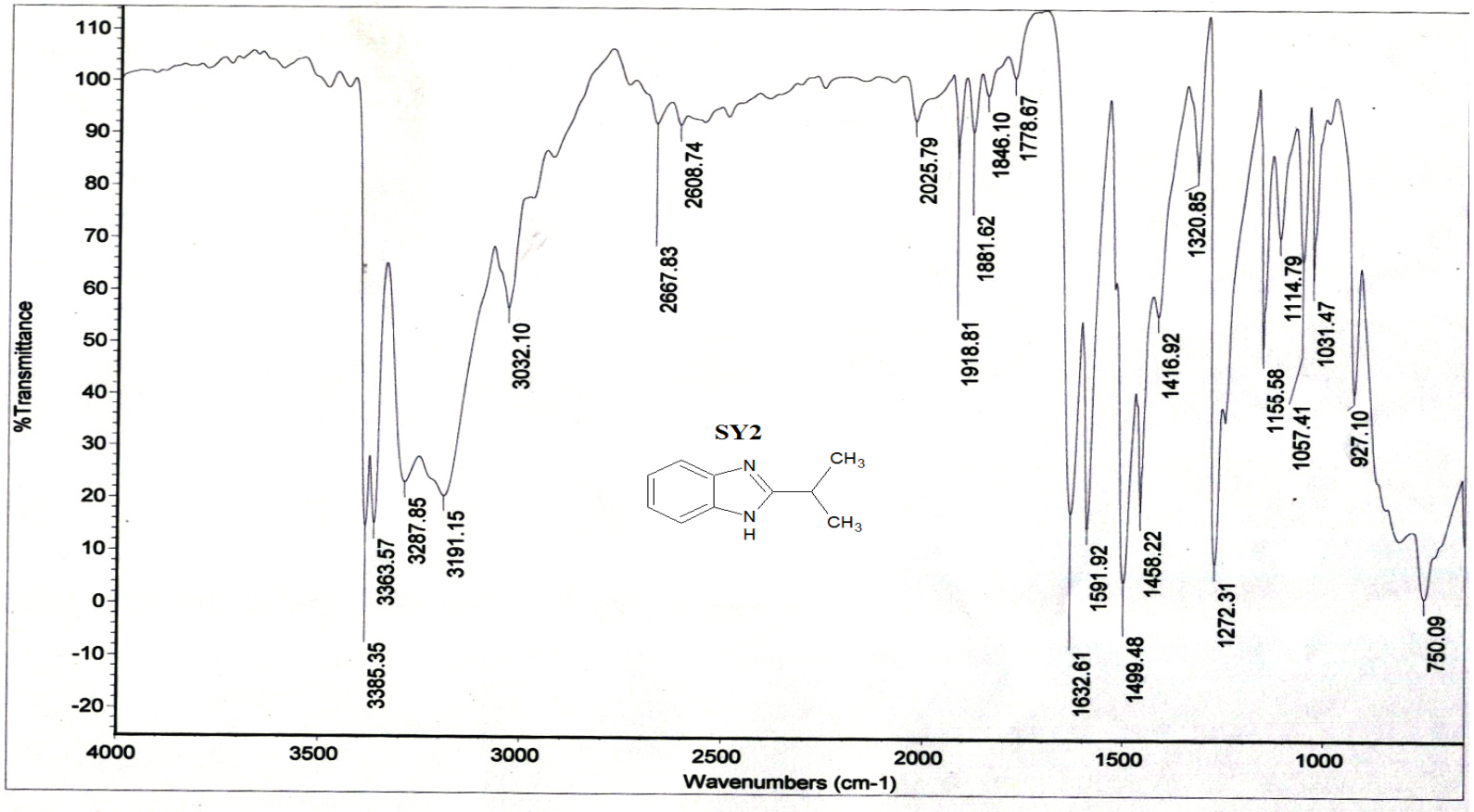


Fig-31: IR Spectrum of compound SY_2

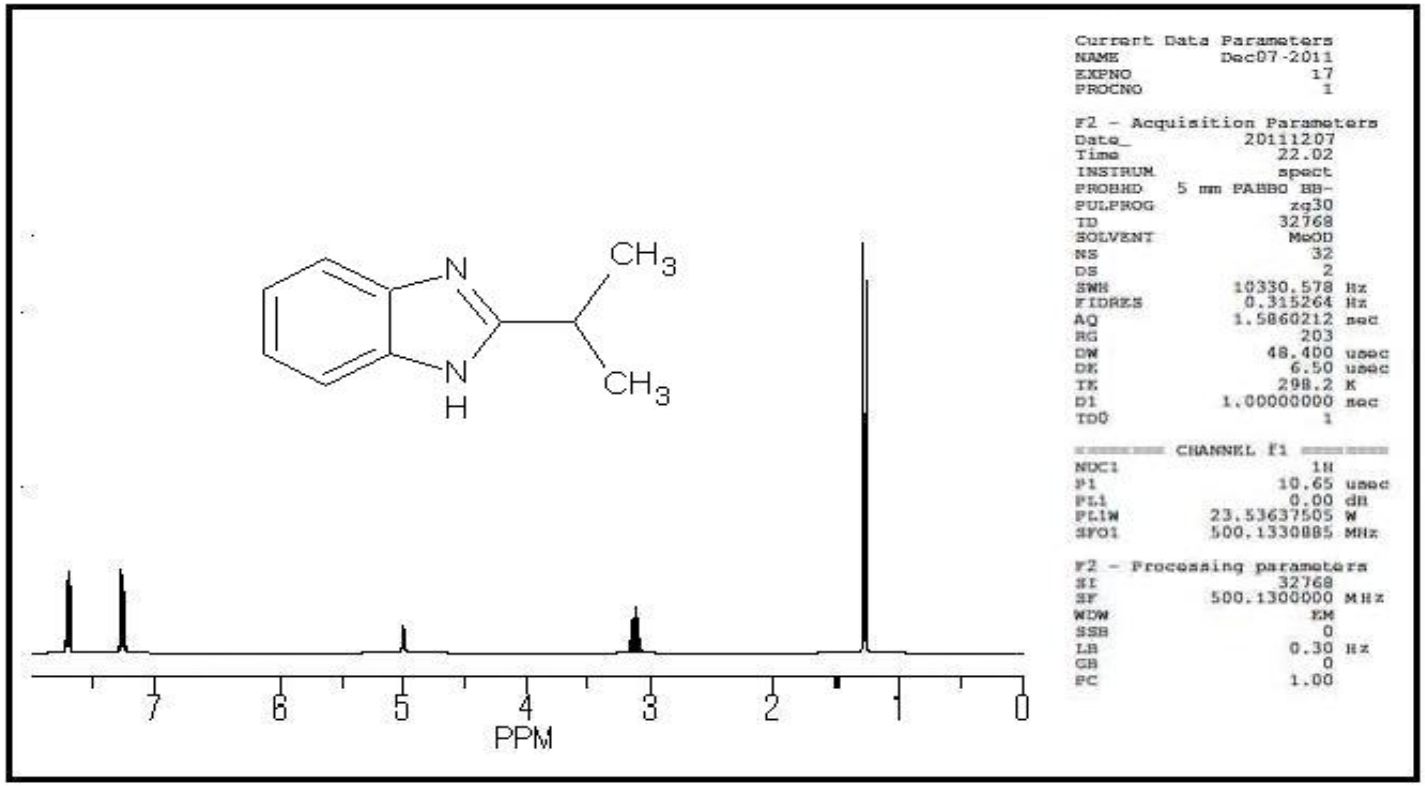


Fig-32: ^{1}H-NMR Spectrum of the compound SY_2

6.2.3. Spectral analysis of 2-butyl-1*H*-benzimidazole:

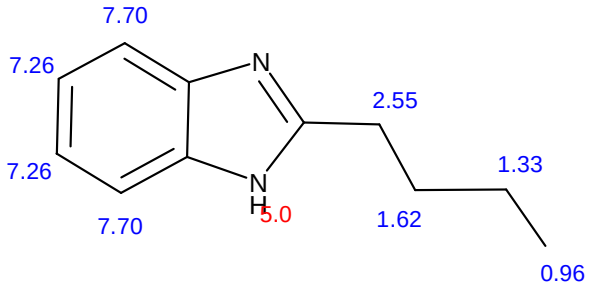
UV: (Fig-33)

λ_{max} (MeOH)	293.5 **(**ε_{max} 0.2321)
λ_{max} (MeOH)	238.0 (ε_{max} 0.4681)

IR (KBr): (Fig-34)

Wave Number (Cm^{-1})	**Assignment**
3385.62	C-H stretching
3192.57	C-H stretching
3288.94	N-H stretching
3032.75	Aromatic C-H stretching
1632.86	C=C stretching
1590.16	C=N stretching
1458.17	C-H bending (scissoring)
1272.45	C-N stretching
1031.54	C-C stretching

NMR (MeOD): (Fig-35)



(4 aromatic protons, 9 aliphatic protons, and 1 proton on nitrogen)

δ	**Assignment**
7.70	(2H, m, Ar-H- C_4&C_7)
7.26	(2H, m, Ar-H- C_5&C_6)
5.0	(1H, s, broad, NH)
2.55	(2H, t, CH_2)
1.62	(2H, m, CH_2)
1.33	(2H, m, CH_2)
0.96	(3H, t, methyl, C_{11})

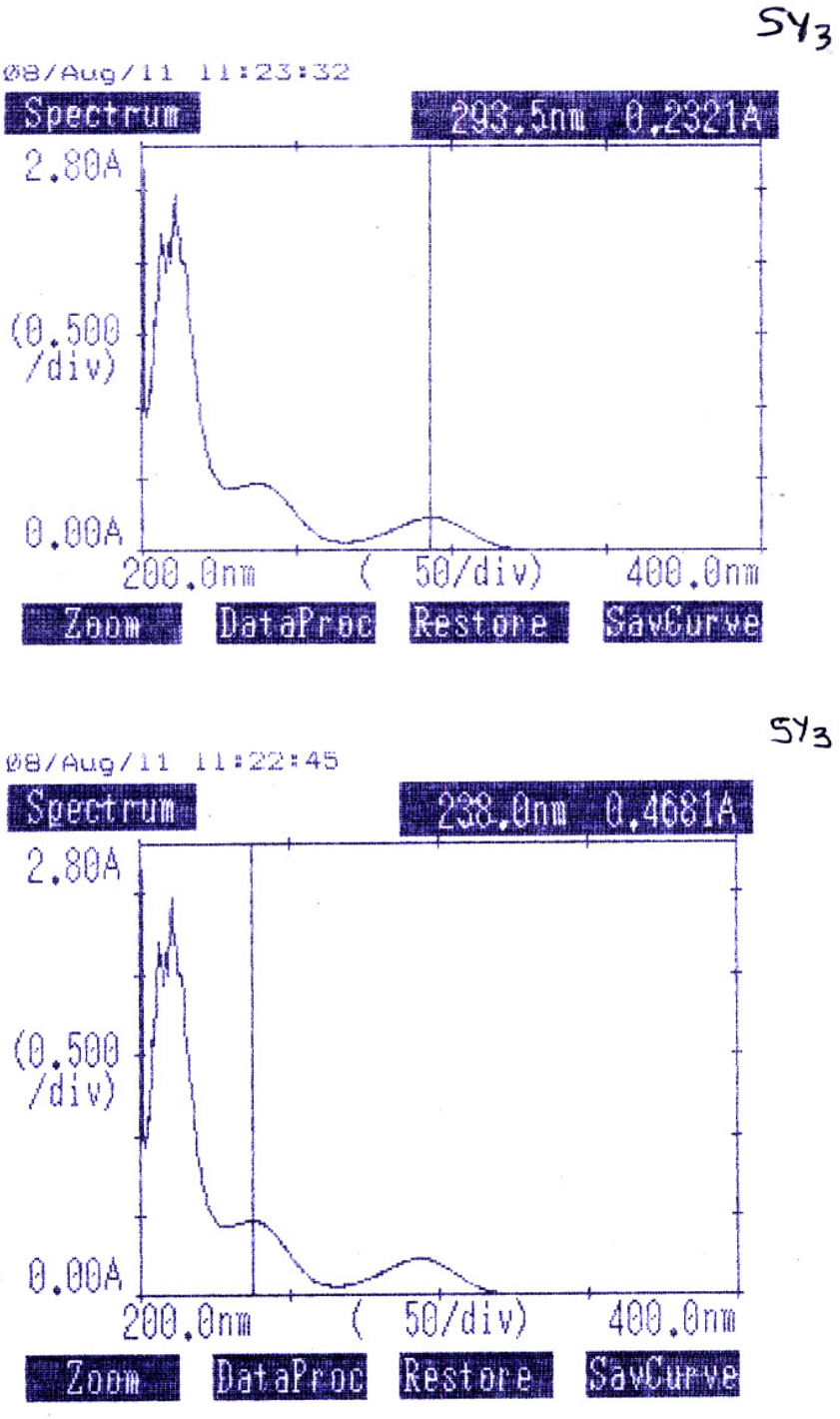


Fig-33: UV Spectrum of compound SY_3

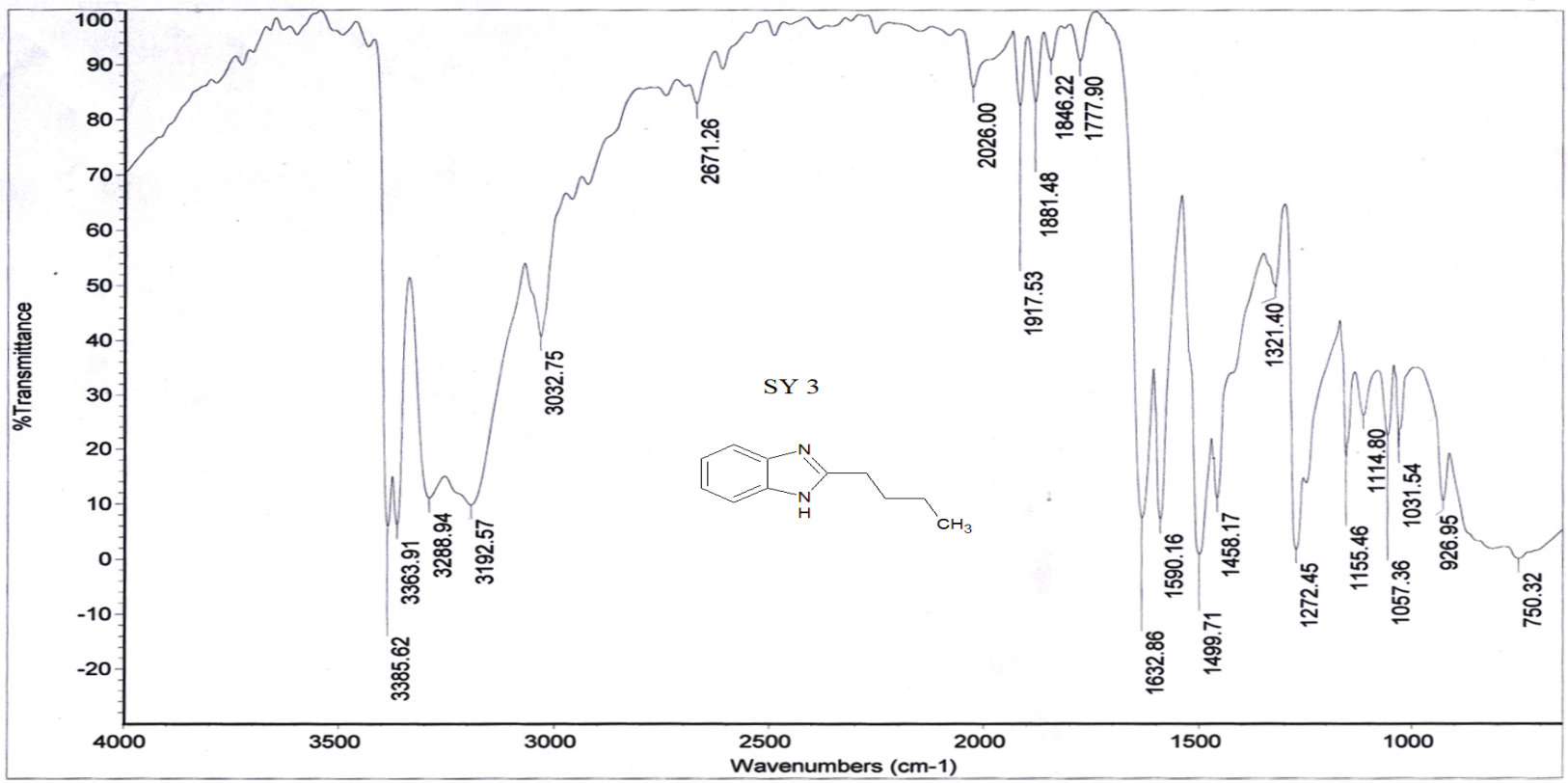


Fig-34: IR Spectrum of compound SY_3

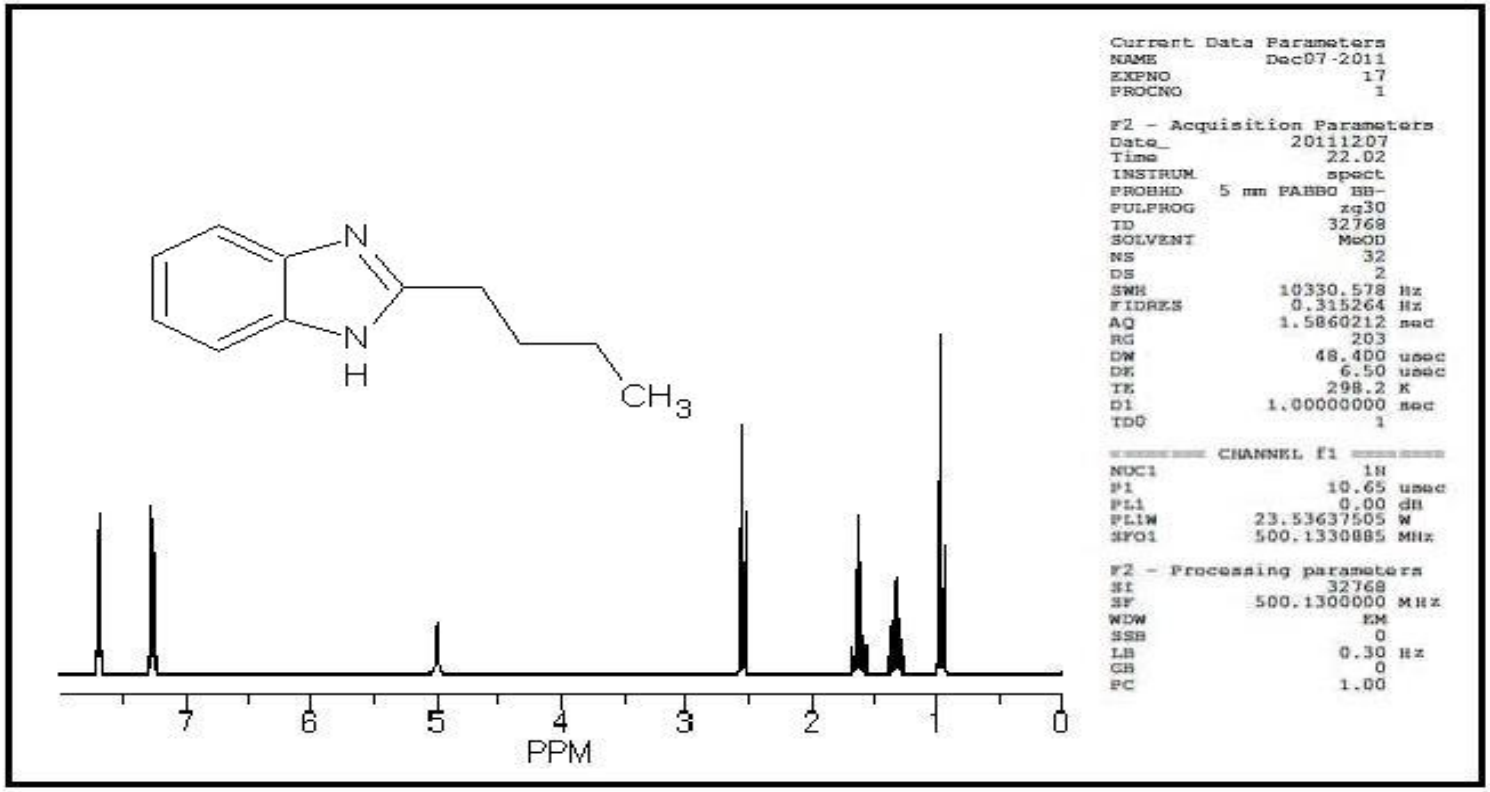


Fig-35: ^{1}H-NMR Spectrumof the Compound SY_3

6.2.4. Spectral analysis of (1*H*-benzimidazol-2-yl) aniline:

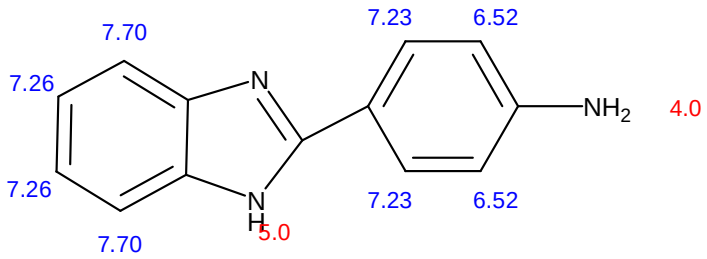
UV: (Fig-36)

λ_{max} (MeOH) 294.5 (ε_{max} 0.4906)

IR (KBr): (Fig-37)

Wave Number (Cm^{-1})	**Assignment**
3514.74	N-H asymmetrical stretching (primary amine)
3463.63	N-H stretching (primary amine)
3358.60	N-H stretching (secondary amine)
2968.78	aromatic C-H stretching
1621.12	N-H bending
1274.47	C-N stretching
1181.99	In plane bending of aromatics
836.02	Out of plane bending of aromatics
697.94	Out of plane bending (substituted benzene)

NMR (MeOD): (Fig-38)



(8 aromatic protons and 3 protons on nitrogen)

δ	**Assignment**
7.70	(2H, m, Ar-H- C_4&C_7)
7.23-7.26	(4H, m, Ar-H- C_5,C_6&$C_{2'}$,$C_{6'}$)
6.52	(2H, m, Ar–H- $C_{3'}$&$C_{5'}$)
5.0	(1H, s, broad, NH)
4.0	(2H, s, NH_2)

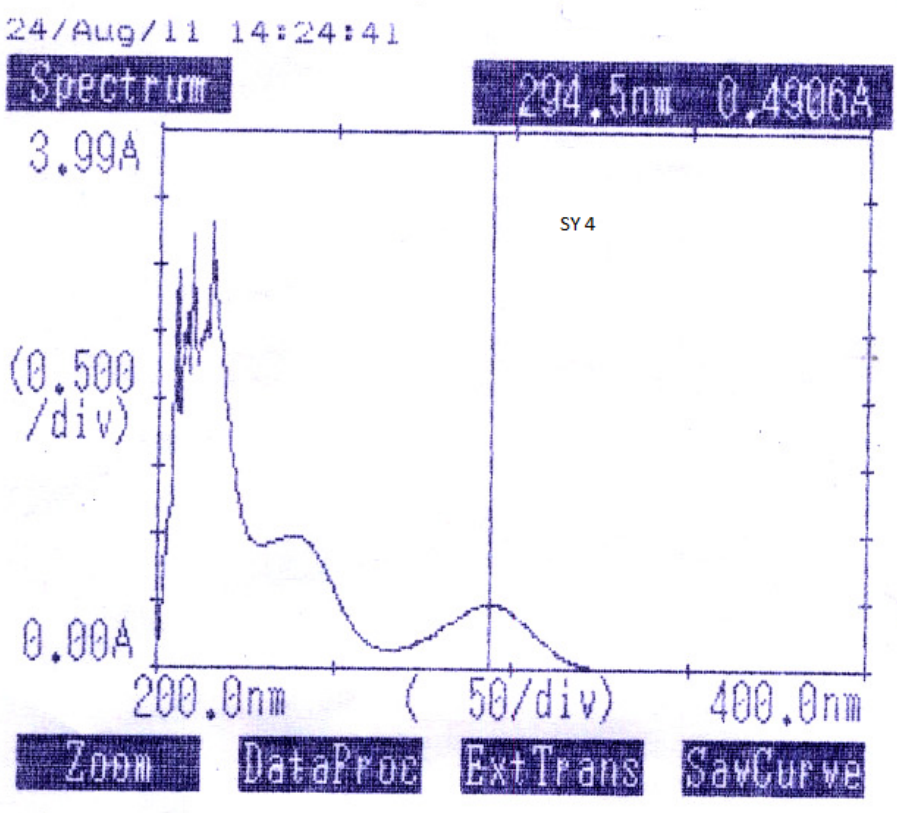


Fig-36: UV Spectrum of compound SY_4

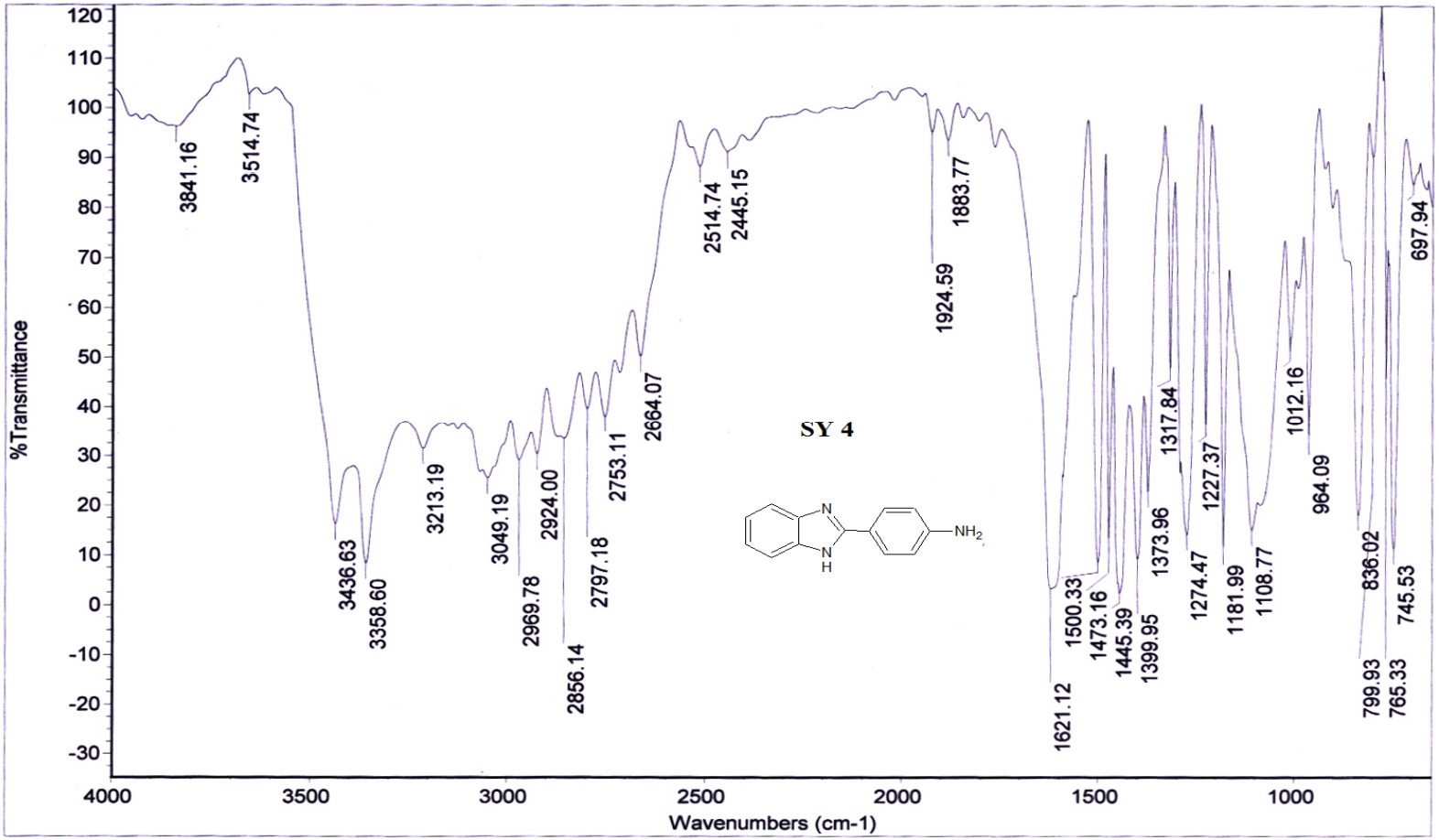


Fig-37: IR Spectrum of compound SY_4

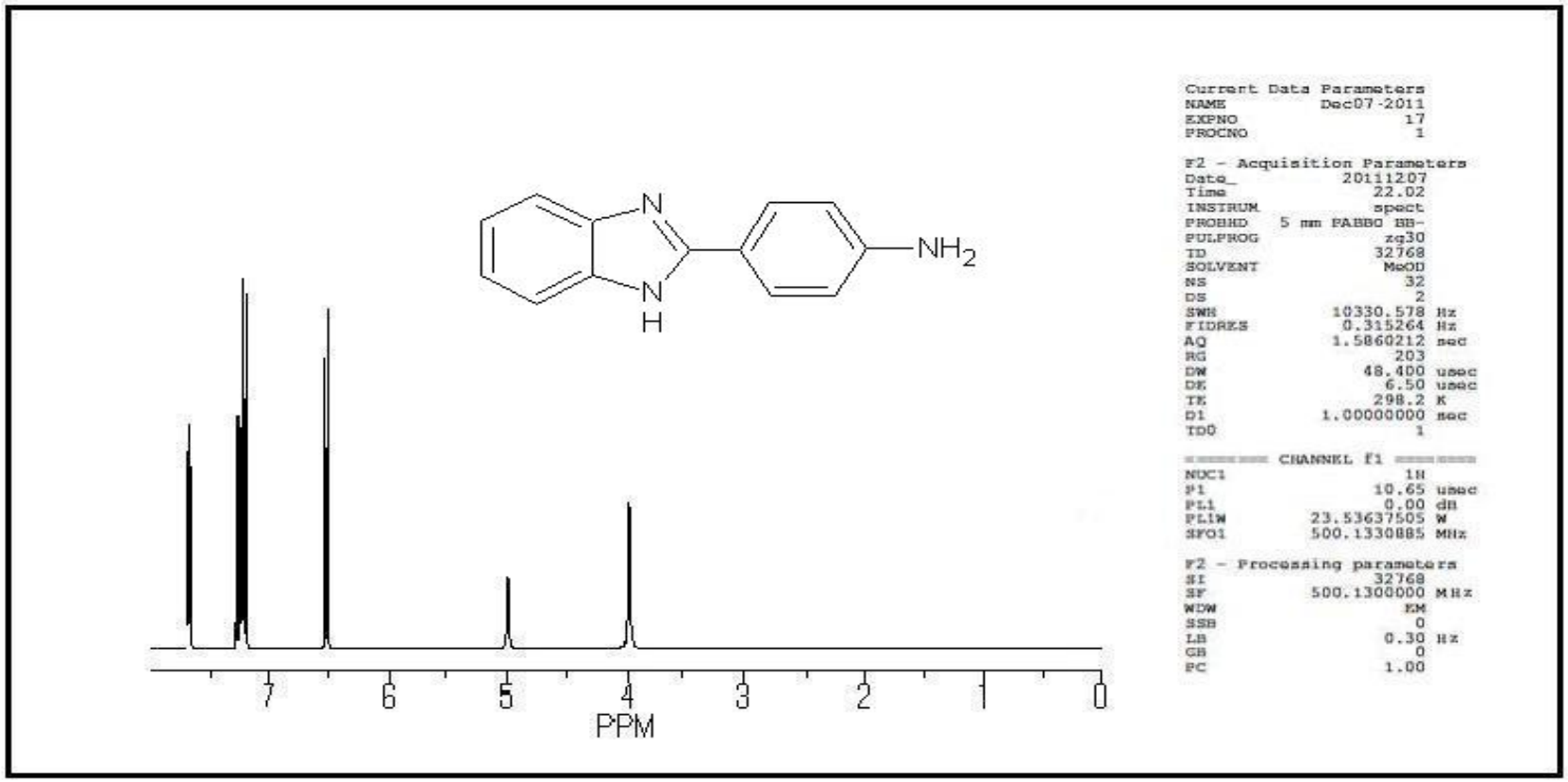


Fig-38: ^{1}H-NMR Spectrum of the compound SY_4

6.2.5. Spectral analysis of 2-(4-nitrophenyl)-1*H*-benzimidazole:

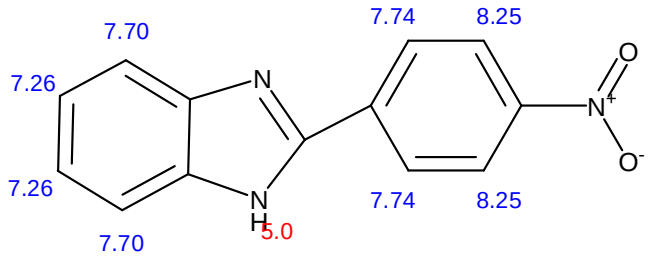
UV: (Fig-39)

λ_{max} (MeOH) 273.0 (ε_{max} 0.4548)

IR (KBr): (Fig-40)

Wave Number (Cm^{-1})	**Assignment**
3114.22	N-H stretching
3062.21	C-H aromatic stretching
1604.17	C-C skeletal stretching
1541.85	N-O asymmetrical stretching
1349.62	N-O symmetrical stretching
1288.31	C-N stretching
1127.45	C-H bending (in plane)
878.93	C-H bending (out plane)

NMR (MeOD): (Fig-41)



(8 aromatic protons and 1 proton on nitrogen)

δ	Assignment
8.25	(2H, m, Ar-H- $C_{3'}$&$C_{5'}$)
7.74	(2H, m, Ar-H- $C_{2'}$&$C_{6'}$)
7.70	(2H, m, Ar-H- C_4&C_7)
7.26	(2H, m, Ar-H- C_5&C_6)
5.0	(1H, s, broad, NH)

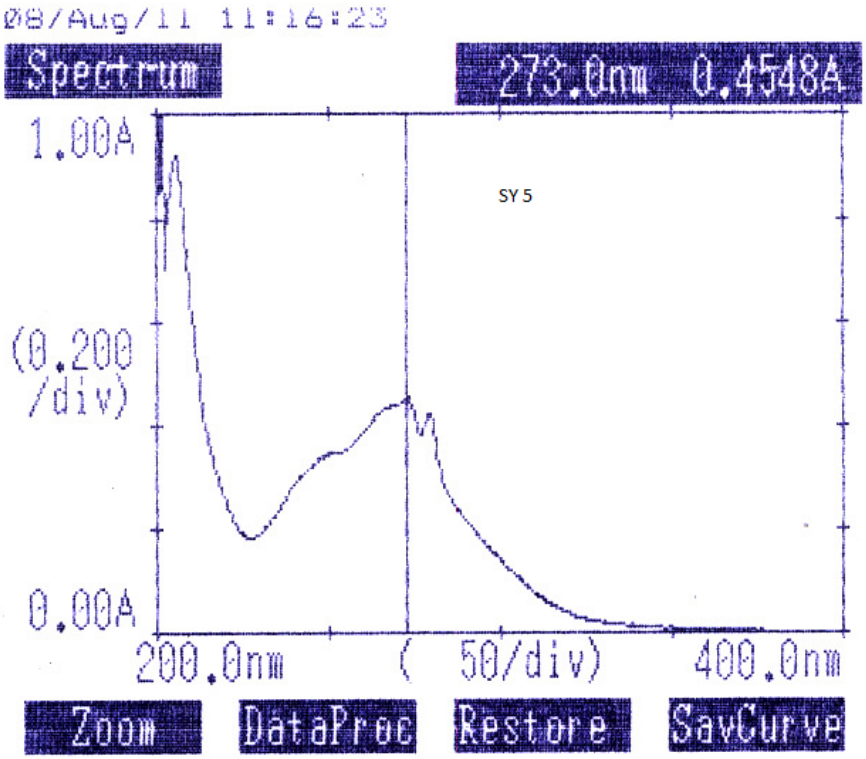


Fig-39: UV Spectrum of compound SY_5

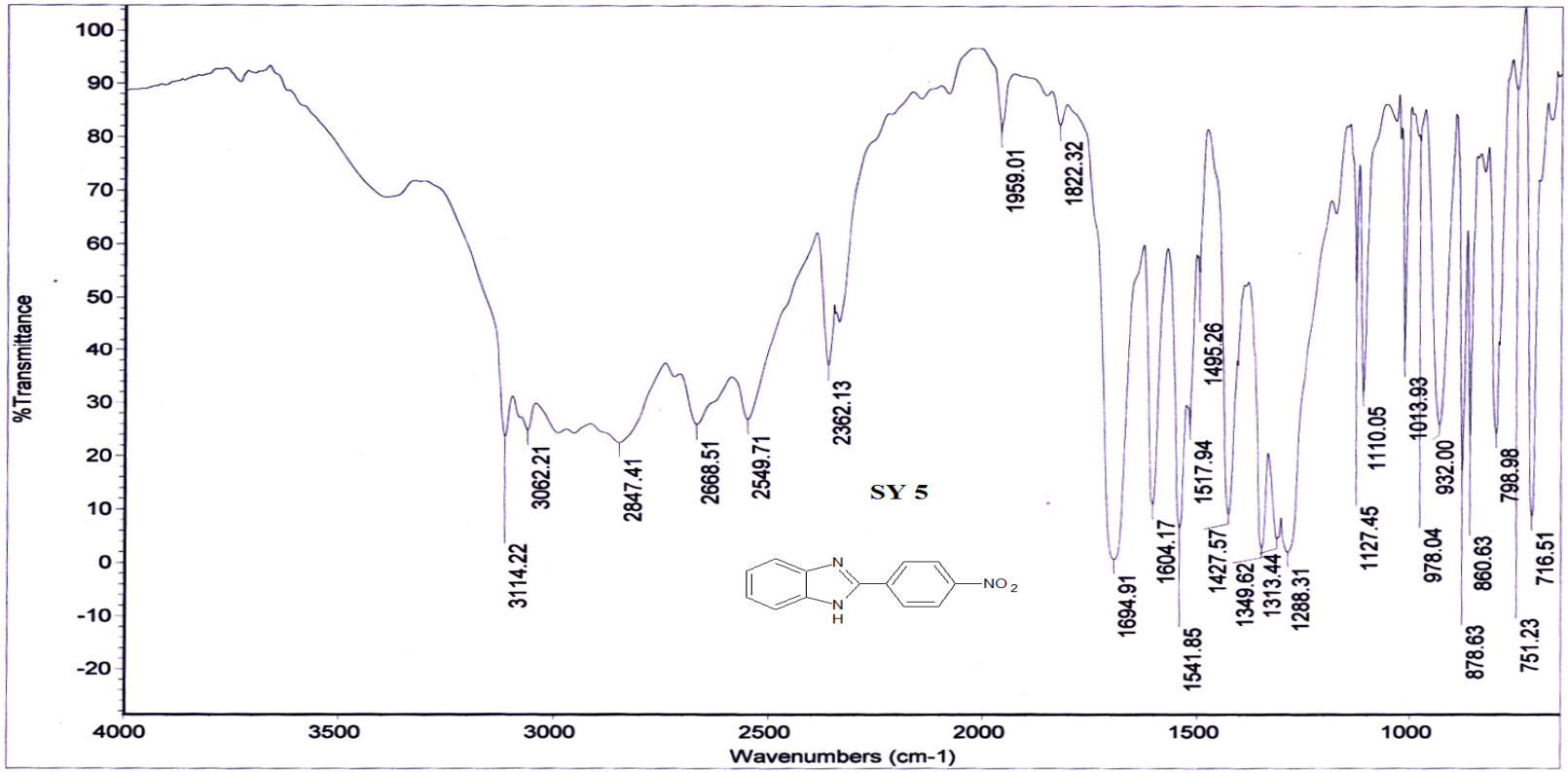


Fig-40: IR Spectrum of compound SY_5

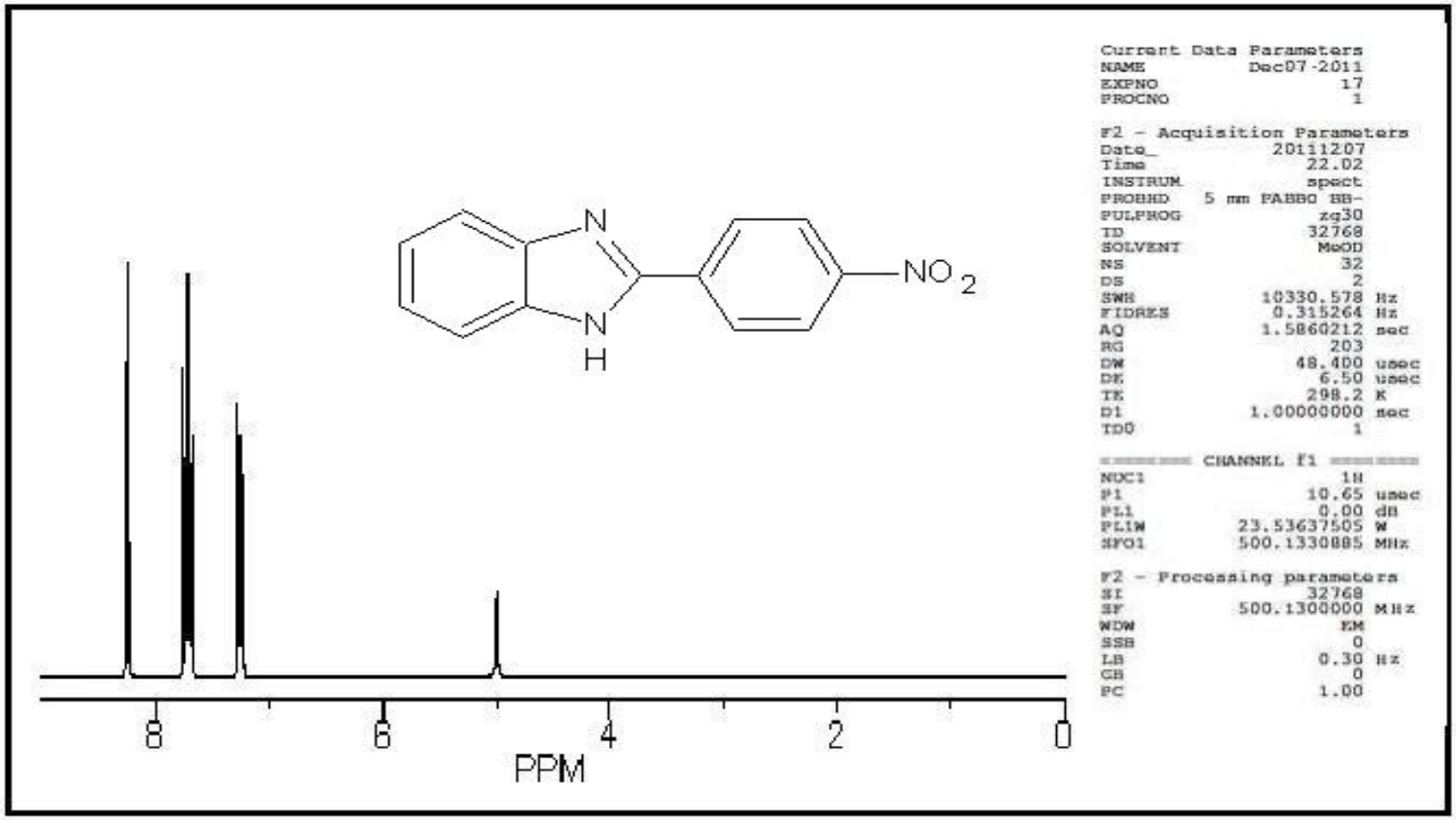


Fig-41: ^{1}H- NMR Spectra of the compound SY_5

6.2.6. Spectral analysis of (5-nitro-1H-benzimidazol-2-yl) methane thiol:

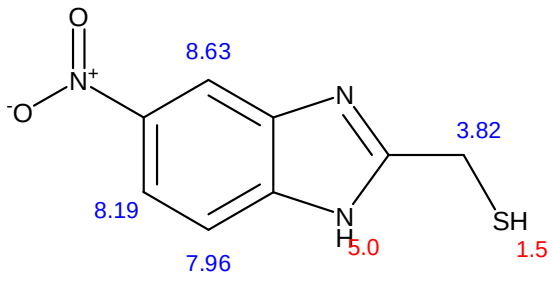
UV: (Fig-42)

λ_{max} (MeOH) 280 .0 (ε_{max} 0.3040)

IR (KBr): (Fig-43)

Wave Number	**Assignment**
3072.23	Aromatic C-H stretching
2933.61	S-H stretching
1683.23	C-C ring stretching
1584.92	N-O asymmetrical stretching
1348.72	N-O symmetrical stretching
1537.17	C=N stretching
846.68	C-N stretching
763.10	N-O bending
715.23	C-S stretching

NMR (MeOD): (Fig-44)



(3 aromatic protons, 2 aliphatic protons, 1 proton on nitrogen and one proton on sulphur)

δ	**Assignment**
8.63	(1H, s, Ar-H- C_4)
8.19	(1H, d, Ar-H- C_6)
7.96	(1H, d, Ar-H- C_7)
5.0	(1H, s, broad, NH)
3.82	(2H, s, CH_2)
1.5	(1H, s, SH)

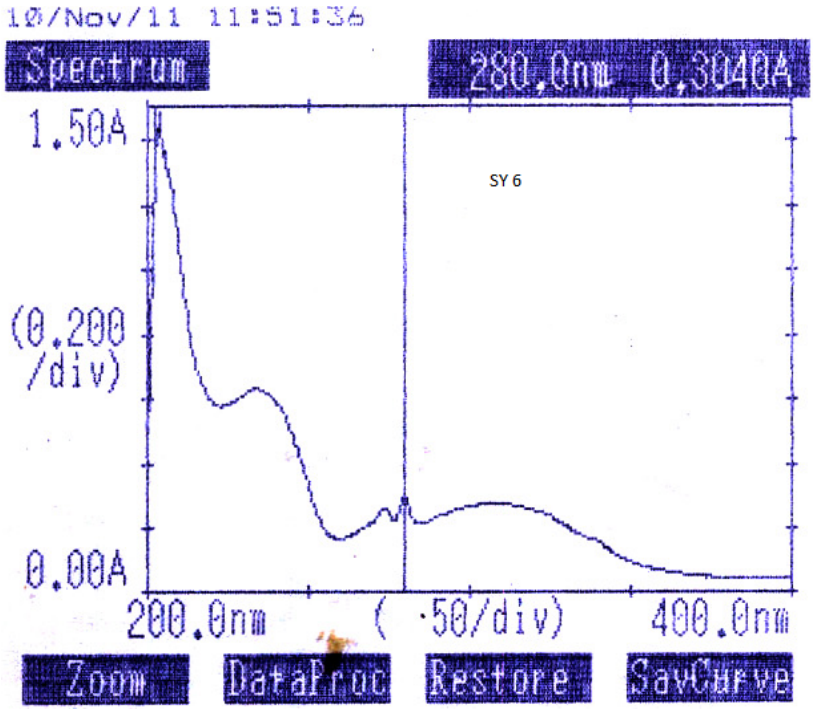


Fig-42 : UV Spectrum of compound SY_6

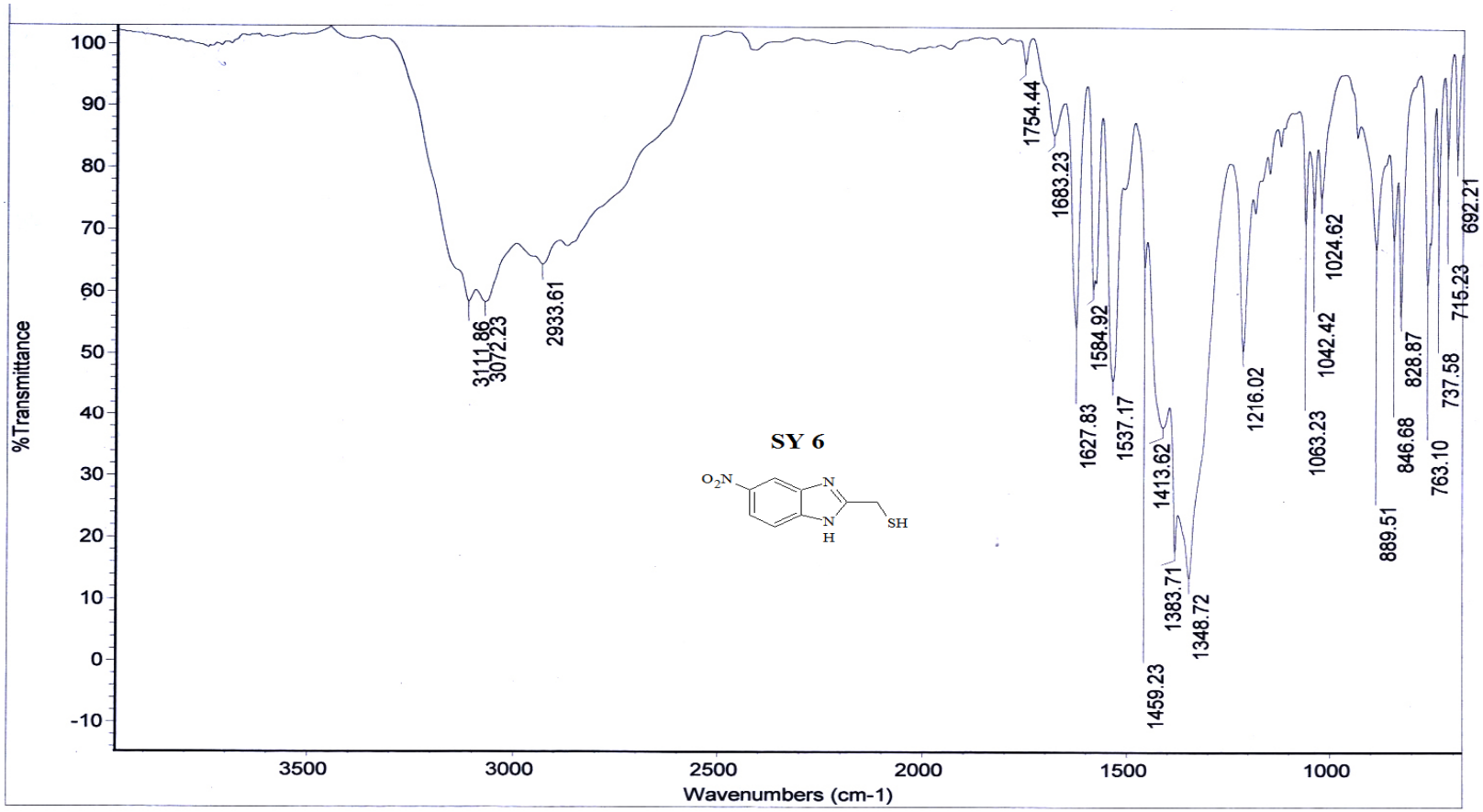


Fig-43: IR Spectrum of compound SY_6

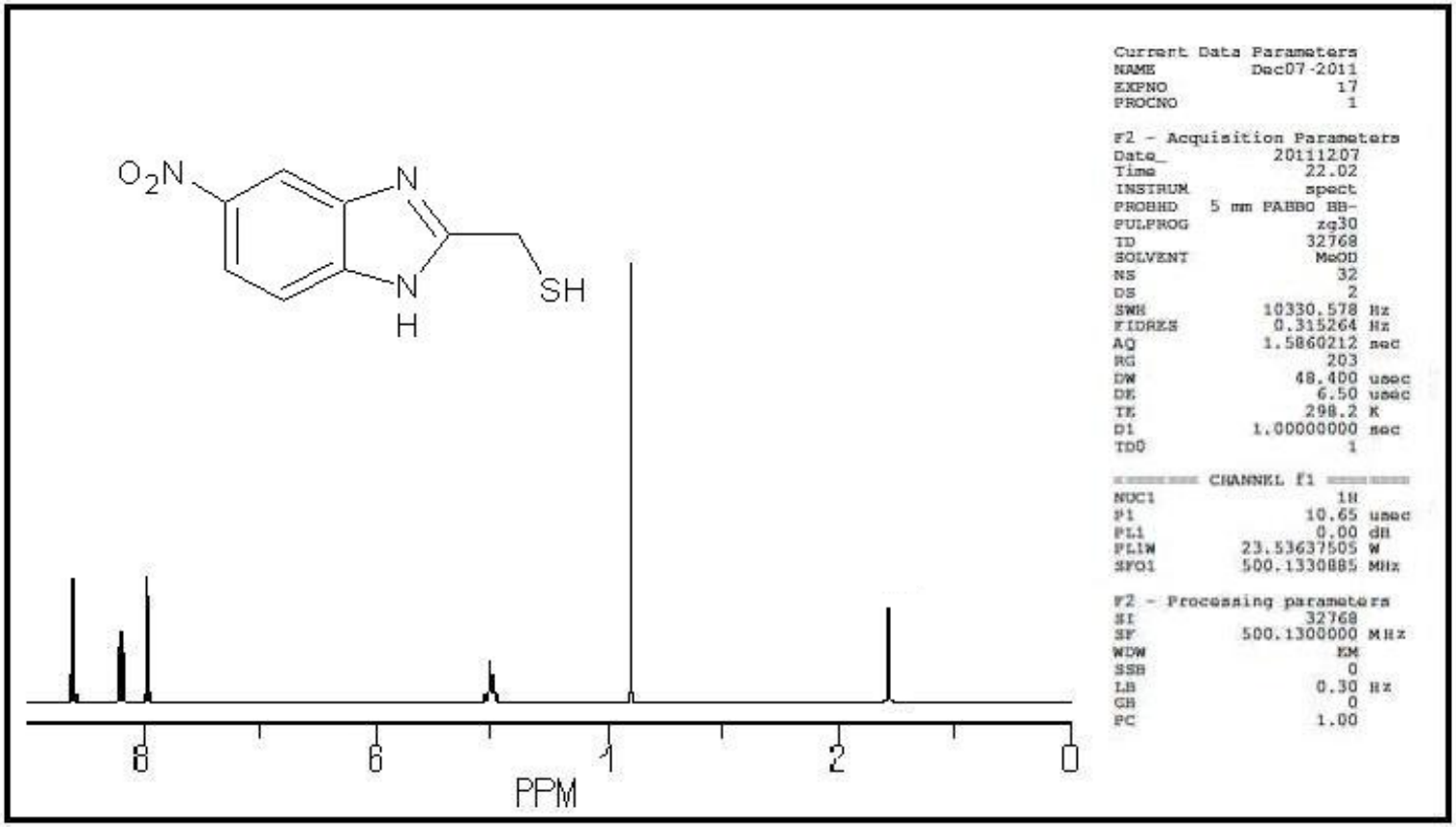


Fig-44: ^{1}H- NMR Spectra of the compound SY_6

6.2.7. Spectral analysis of 5-nitro 2-(propan-2-yl)-1*H*-benzimidazole:

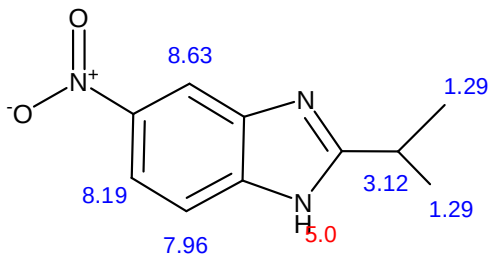
UV: (Fig-45)

λ_{max} (MeOH)	233.0 (ε_{max} 0.1938)

IR (KBr): (Fig-46)

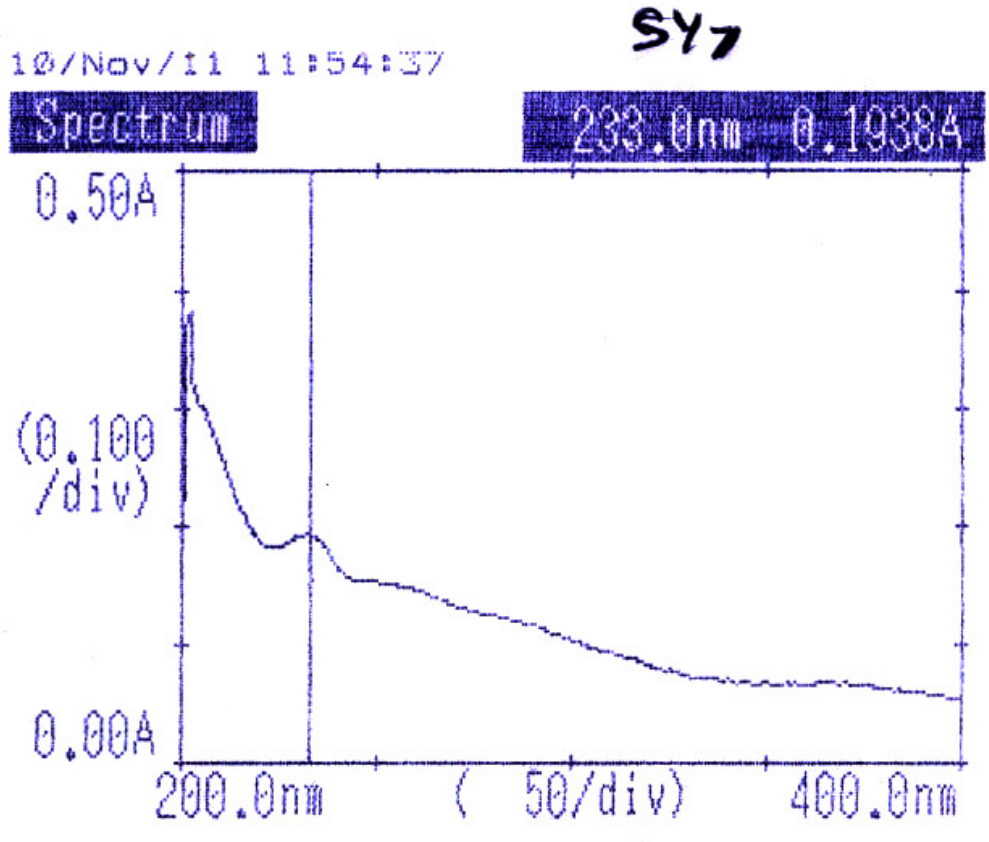
Wave Number	**Assignment**
3384.19	N-H stretching
3098.25	Aromatic C-H stretching
1612.71	N-H bending
1538.03	N-O asymmetrical stretching
1348.03	N-O symmetrical stretching
1217.67	C-N stretching
883.21	C-C stretching
755.96	C-H bending

NMR (MeOD): (Fig-47)



(3 aromatic protons, 7 aliphatic protons, and 1 proton on nitrogen)

δ	Assignment
8.63	(1H, s, Ar-H- C_4)
8.19	(1H, d, Ar-H- C_6)
7.96	(1H, d, Ar-H- C_7)
5.0	(1H, s, broad, NH)
3.12	(1H, m, CH of isopropyl)
1.29	(6H, d, $2CH_3$ of isopropyl)



(Fig-45 : UV Spectrum of compound SY_7)

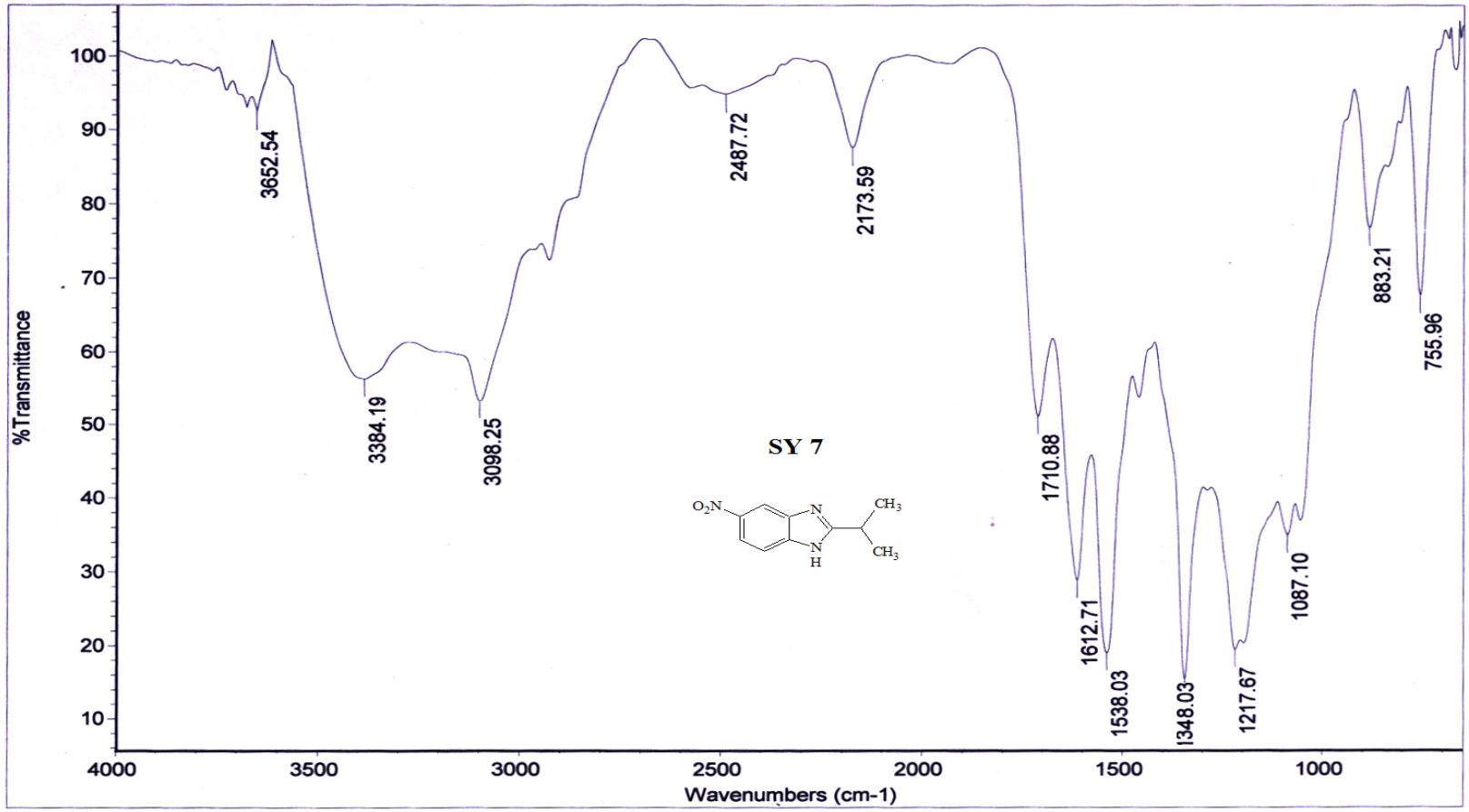


Fig-46: IR Spectrum of compound SY_7

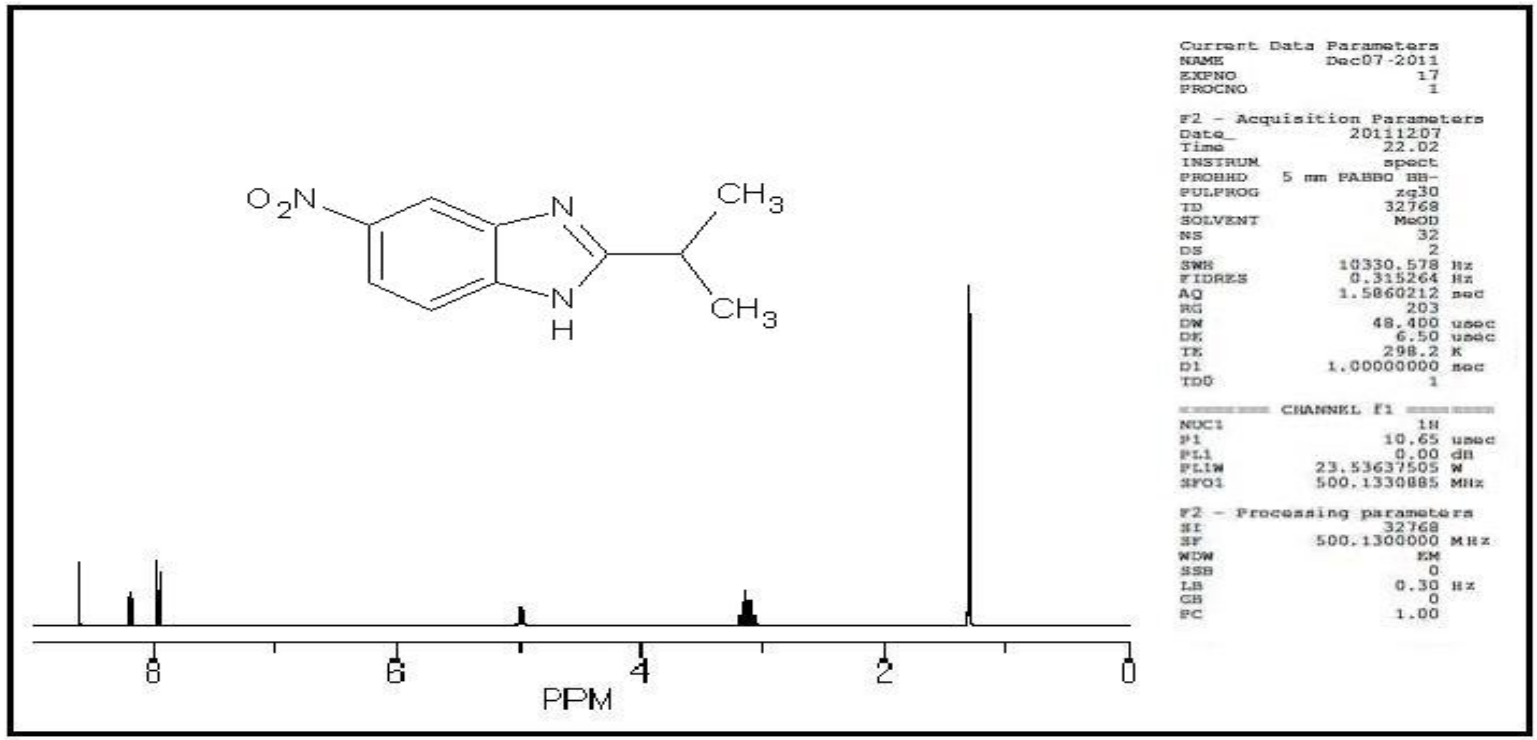


Fig 47: ^{1}H- NMR Spectra of the compound SY_7

6.2.8. Spectral analysis of 5-nitro 2-butyl-1*H*-benzimidazole:

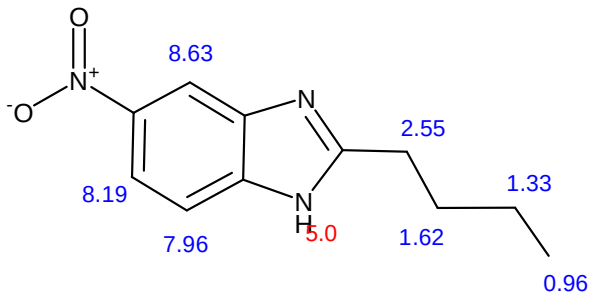
UV: (Fig-48)

λ_{max} (MeOH) 244.0 (ε_{max} 2.3824)

IR (KBr): (Fig-49)

Wave Number	**Assignment**
3651.02	N-H stretching
3098.41	Aromatic C-H stretching
1541.67	N-O asymmetrical stretching
1344.16	N-O symmetrical stretching
1456.09	C-H bending
1209.52	C-N stretching
892.90	C-C stretching
751.82	C-H bending (Wagging)

NMR (MeOD): (Fig-50)



(3 aromatic protons, 9 aliphatic protons, and 1 proton on nitrogen)

δ	Assignment
8.63	(1H, s, Ar-H- C_4)
8.19	(1H, d, Ar-H- C_5)
7.96	(1H, d, Ar-H- C_7)
5.0	(1H, s, broad, NH)
2.55	(2H, t, CH_2)
1.62	(2H, m, CH_2)
1.33	(2H, m, CH_2)
0.96	(3H, t, CH_3)

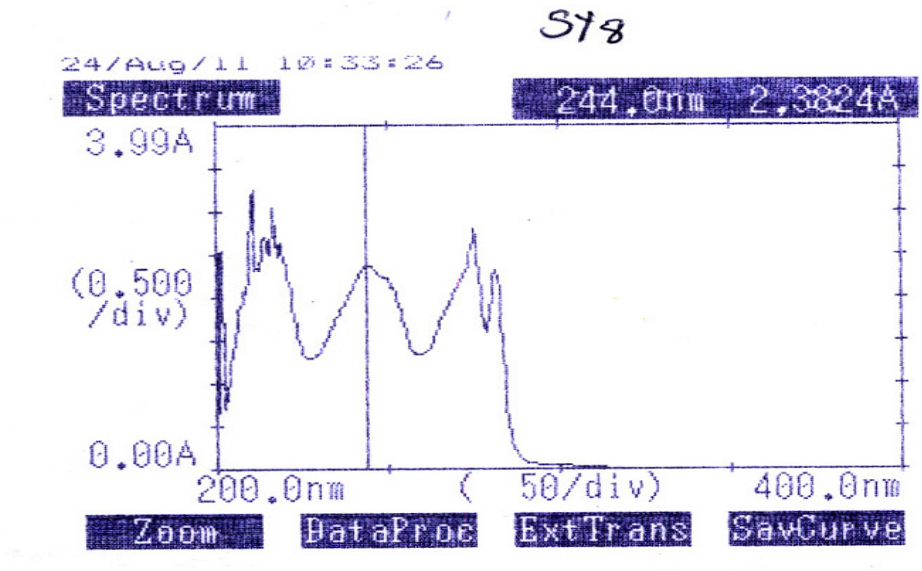


Fig- 48: UV Spectrum of compound SY_8

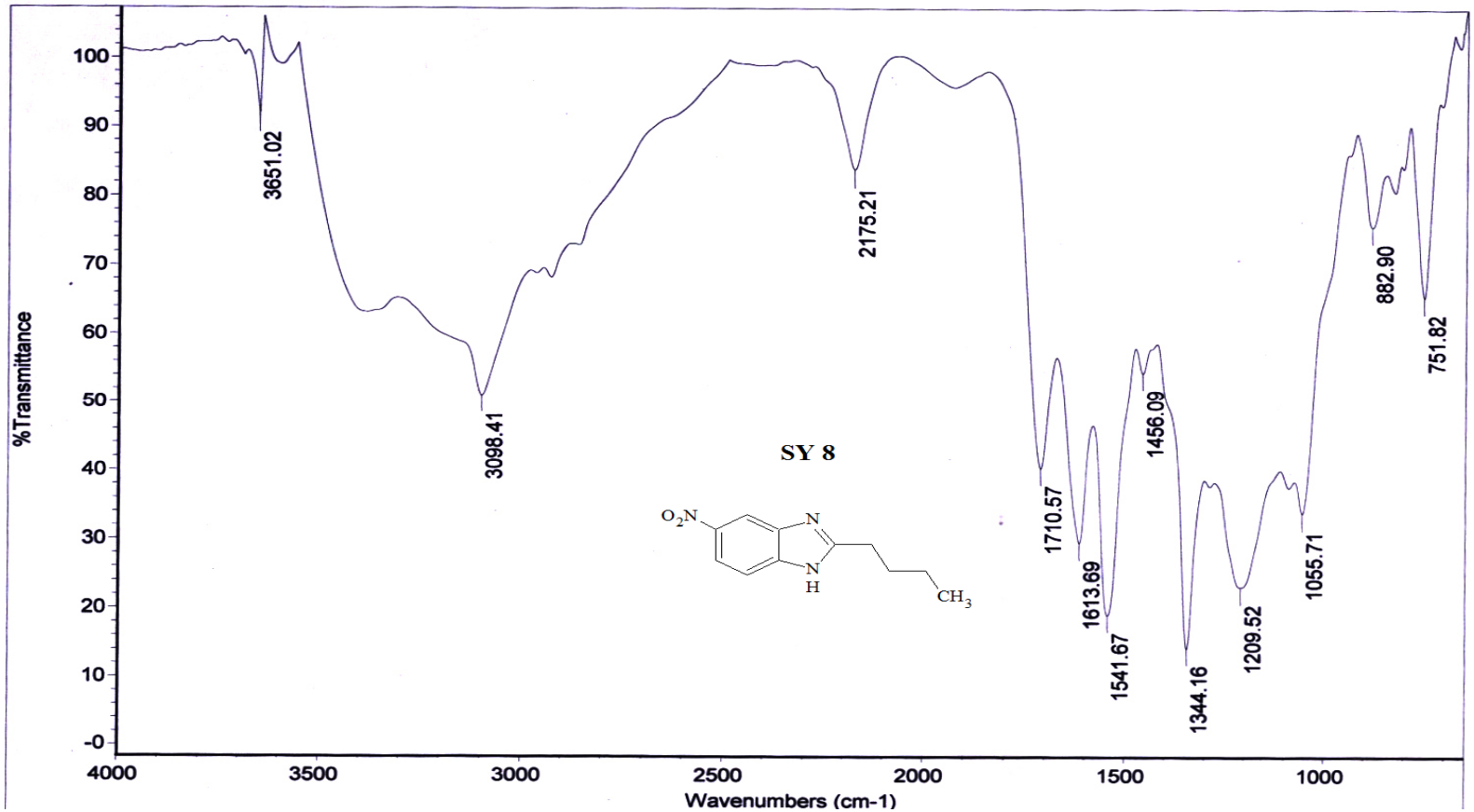


Fig-49: IR Spectrum of compound SY_8

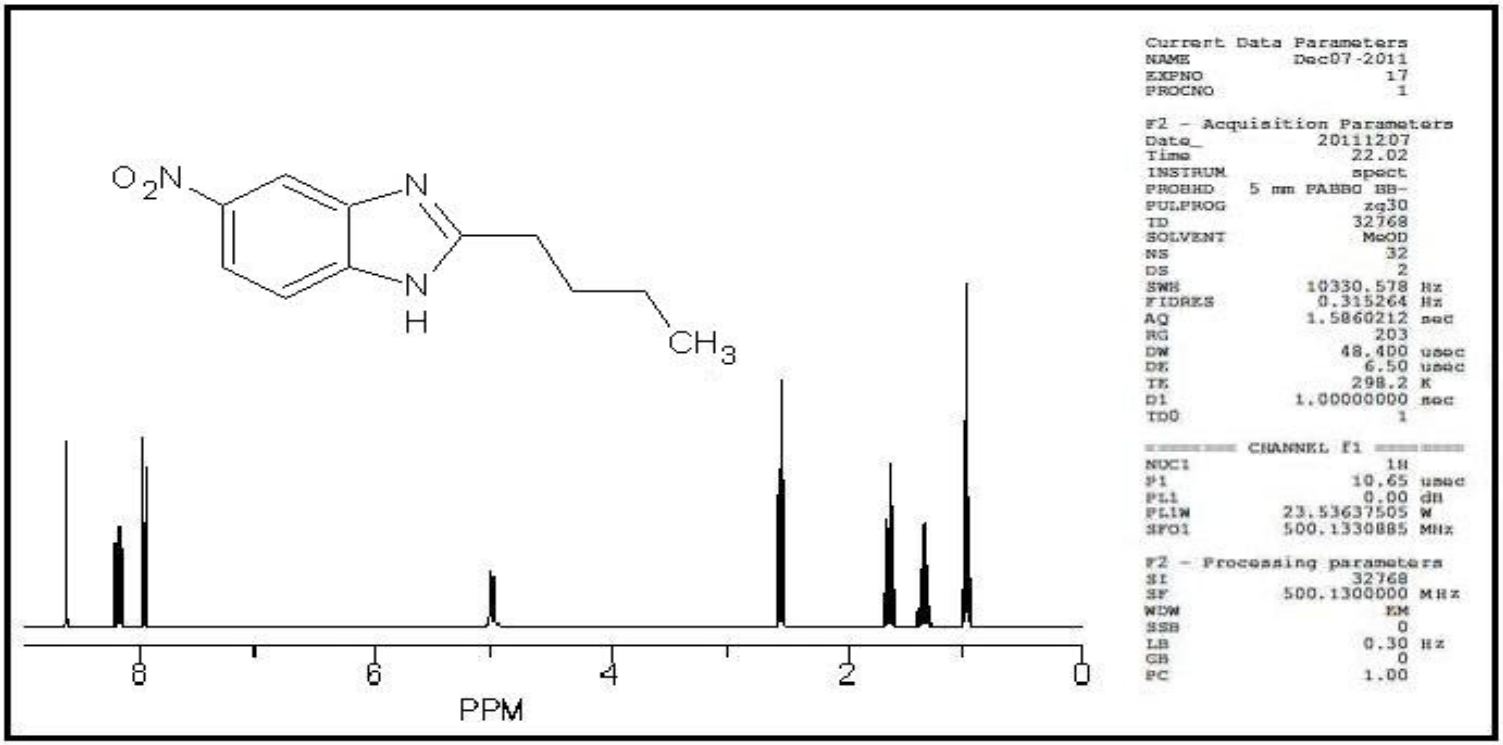


Fig-50: ^{1}H-NMR Spectra of the compound SY_8

6.2.9. Spectral analysis of 4-(5-nitro-1*H*-benzimidazol-2-yl) aniline:

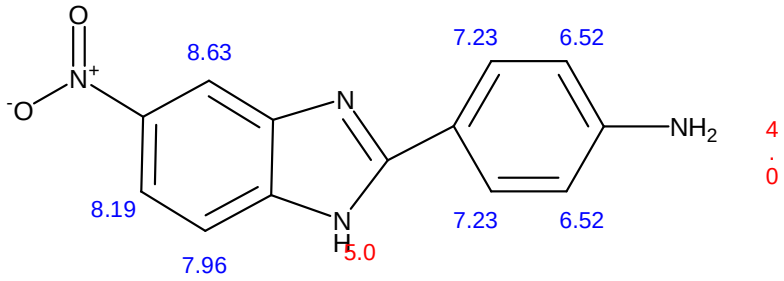
UV: (Fig-51)

λ_{max} (MeOH) 313.0 (ε_{max} 0.3320)

IR (KBr): (Fig-52)

Wave Number	**Assignment**
3418.50	N-H symmetrical stretching (primary amine)
3322.70	N-H stretching (secondary amine)
3072.36	Aromatic C-H stretching
1644.22	N-H bending
1532.92	N-O asymmetrical stretching
1344.12	N-O symmetrical stretching
1231.34	C-N stretching

NMR (MeOD): (Fig-53)



(7 aromatic protons and three protons on nitrogen)

δ	Assignment
8.63	(1H, s, Ar-H- C_4)
8.19	(1H, d, Ar-H- C_6)
7.96	(1H, d, Ar-H- C_7)
7.23	(2H, m, Ar-H- $C_{2'}$&$C_{6'}$)
6.52	(2H, m, Ar-H- $C_{3'}$&$C_{5'}$)
5.0	(1H, s, broad, NH)
4.0	(2H, s, NH_2)

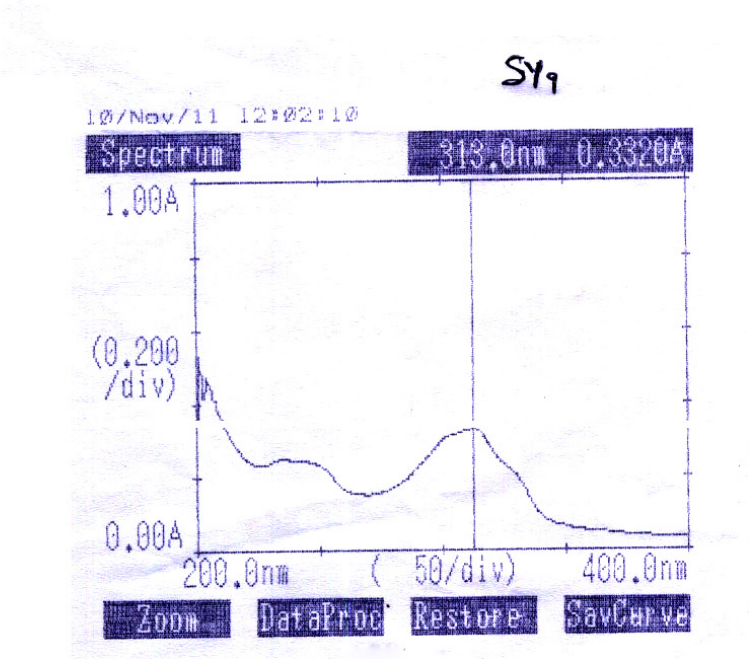


Fig-51: UV spectrum of compound SY_9

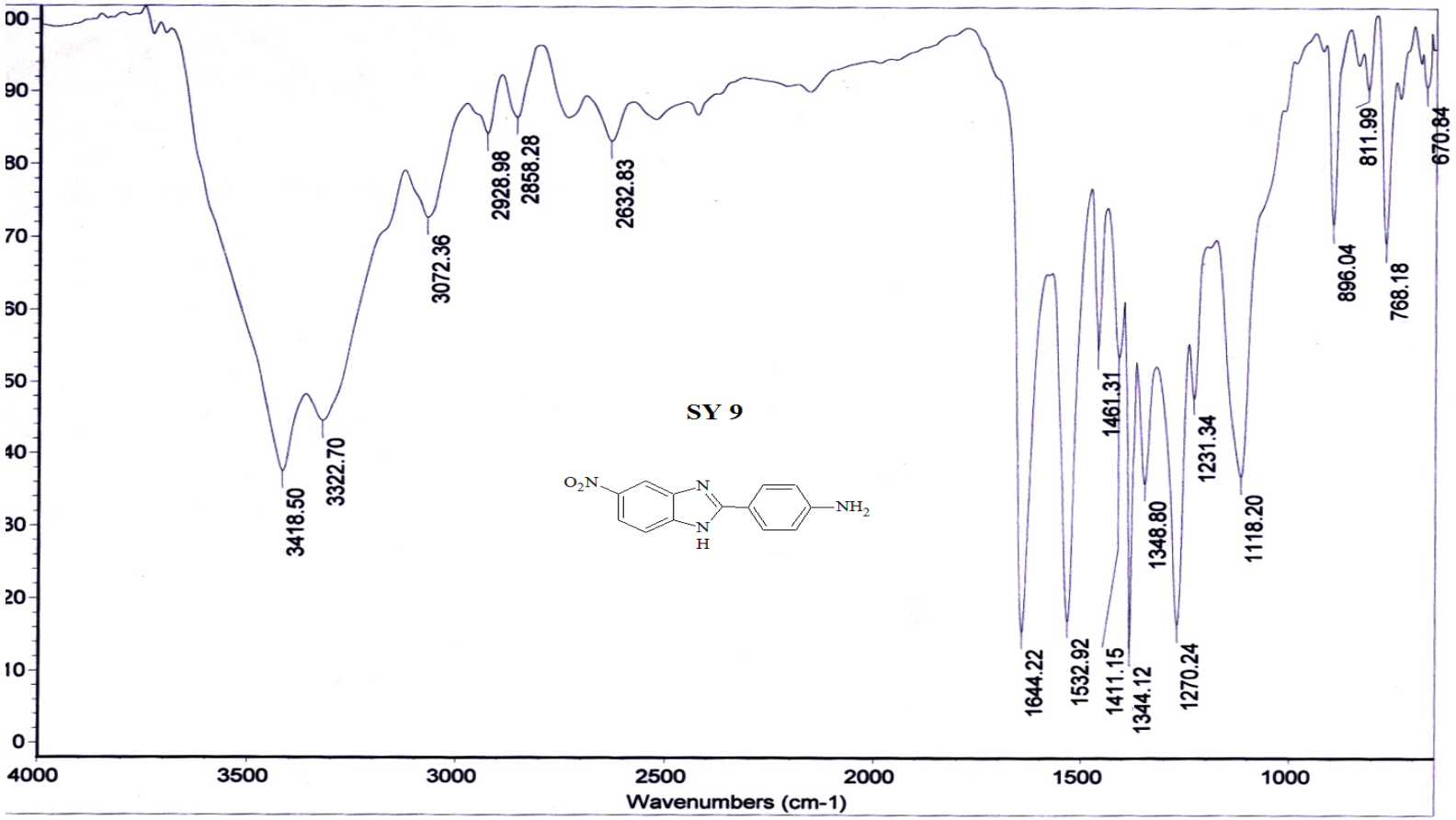


Fig-52: IR Spectrum of compound SY_9

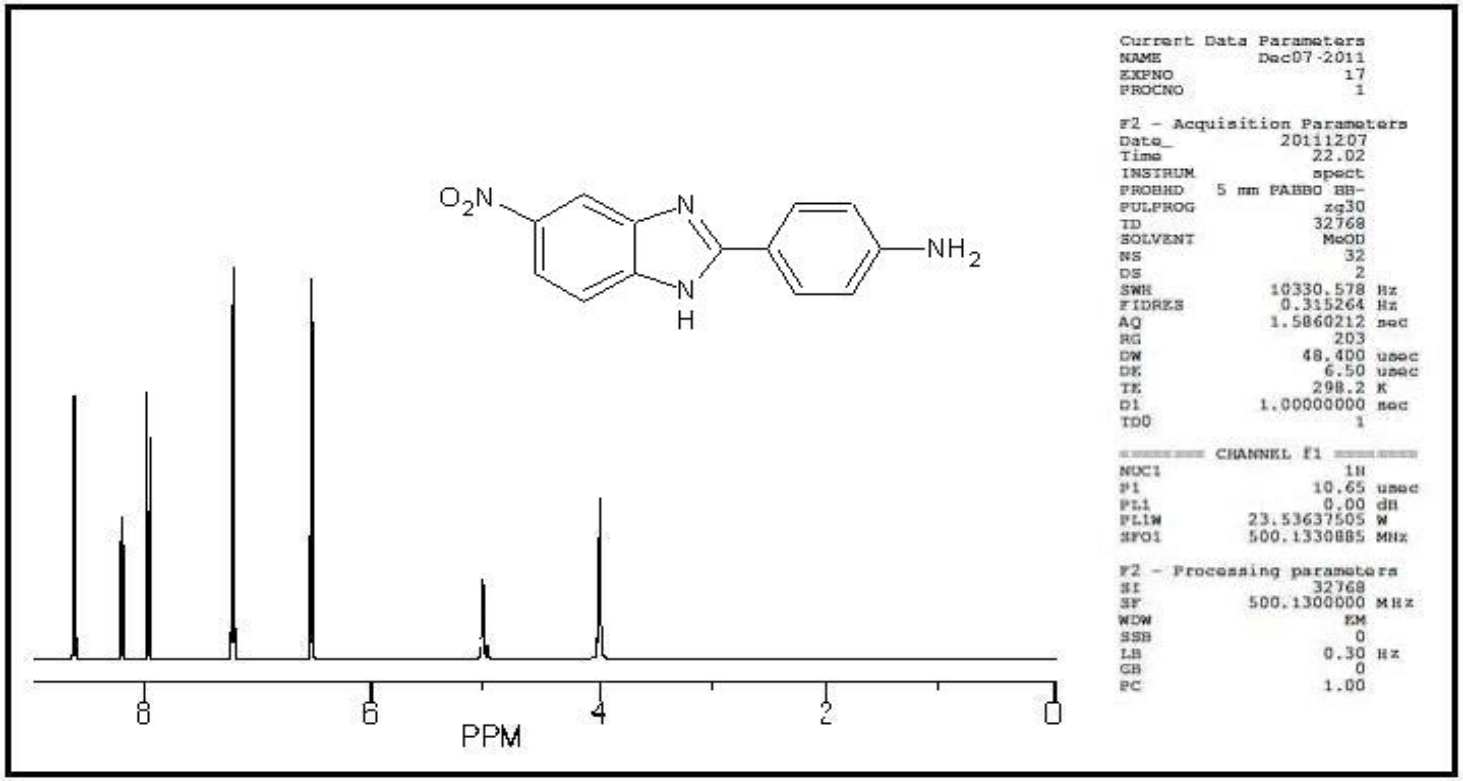


Fig-53: ^{1}H- NMR Spectra of the compound SY_9

6.2.10. Spectral analysis of 5-nitro 2-(4-nitrophenyl)-1*H*-benzimidazole:

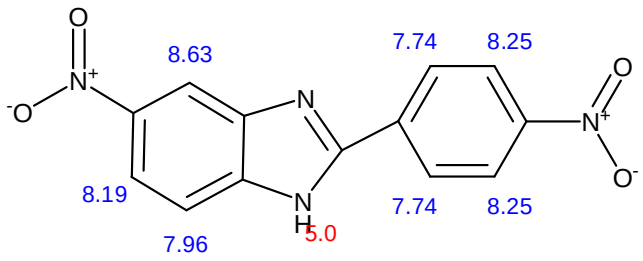
UV: (Fig-54)

λ_{max} (MeOH)	267.5 (ε_{max} 0.9932)

IR (KBr): (Fig-55)

Wave Number (Cm^{-1})	**Assignment**
3114.32	N-H stretching
3061.87	Aromatic C-H stretching
1698.28	N-O asymmetrical stretching
1604.29	N-H bending
1349.69	N-O symmetrical stretching
1129.71	In plane bending of aromatics
877.69	C-N stretching (nitro aromatics)
859.46	Out of plane bending of aromatics

NMR (MeOD): (Fig-56)



(7 aromatic protons and 1 proton on nitrogen)

δ	Assignment
8.63	(1H, s, Ar-H- C_4)
8.25	(2H, m, Ar-H- $C_{3'}$&$C_{5'}$)
8.19	(1H, d, Ar-H- C_6)
7.96	(1H, d, Ar-H- C_7)
7.74	(2H, m, Ar-H- $C_{2'}$&$C_{6'}$)
5.0	(1H, s, broad, NH)

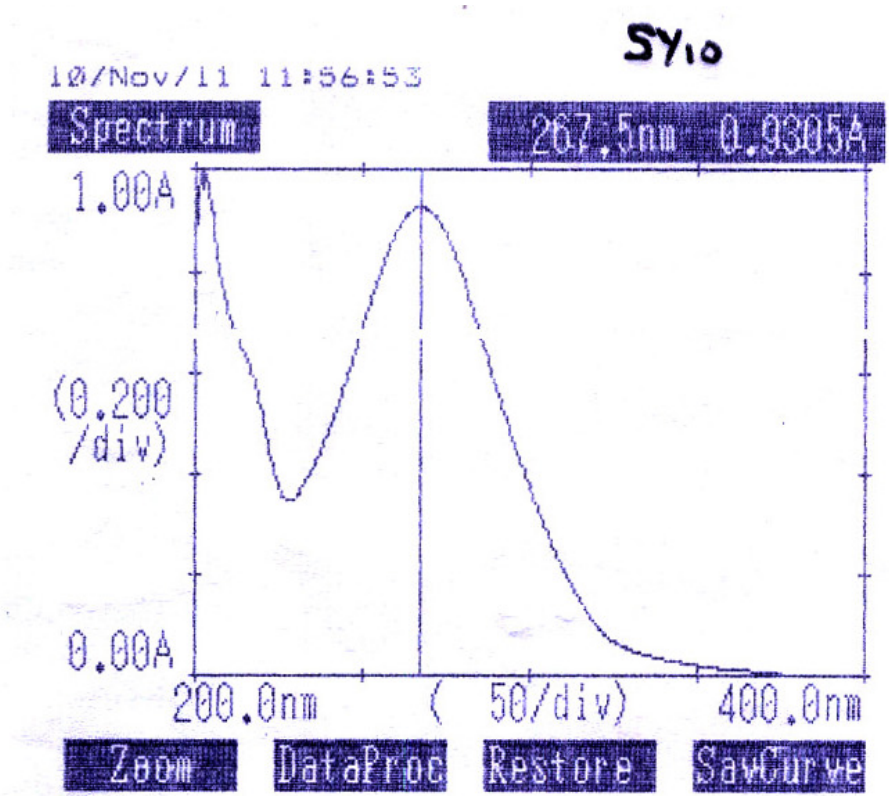


Fig-54: UV Spectrum of compound SY_{10}

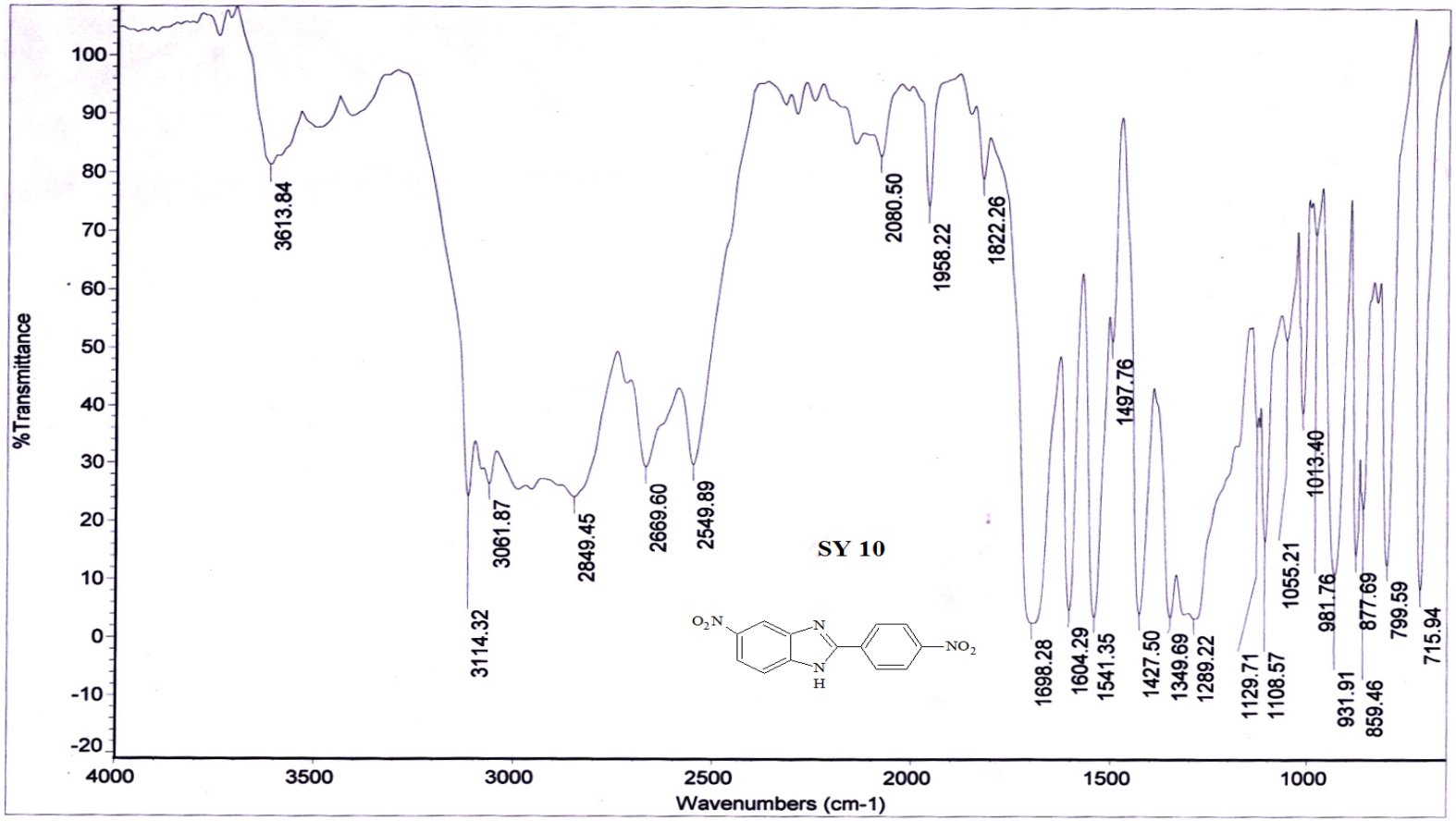


Fig-55: IR Spectrum of compound SY_{10}

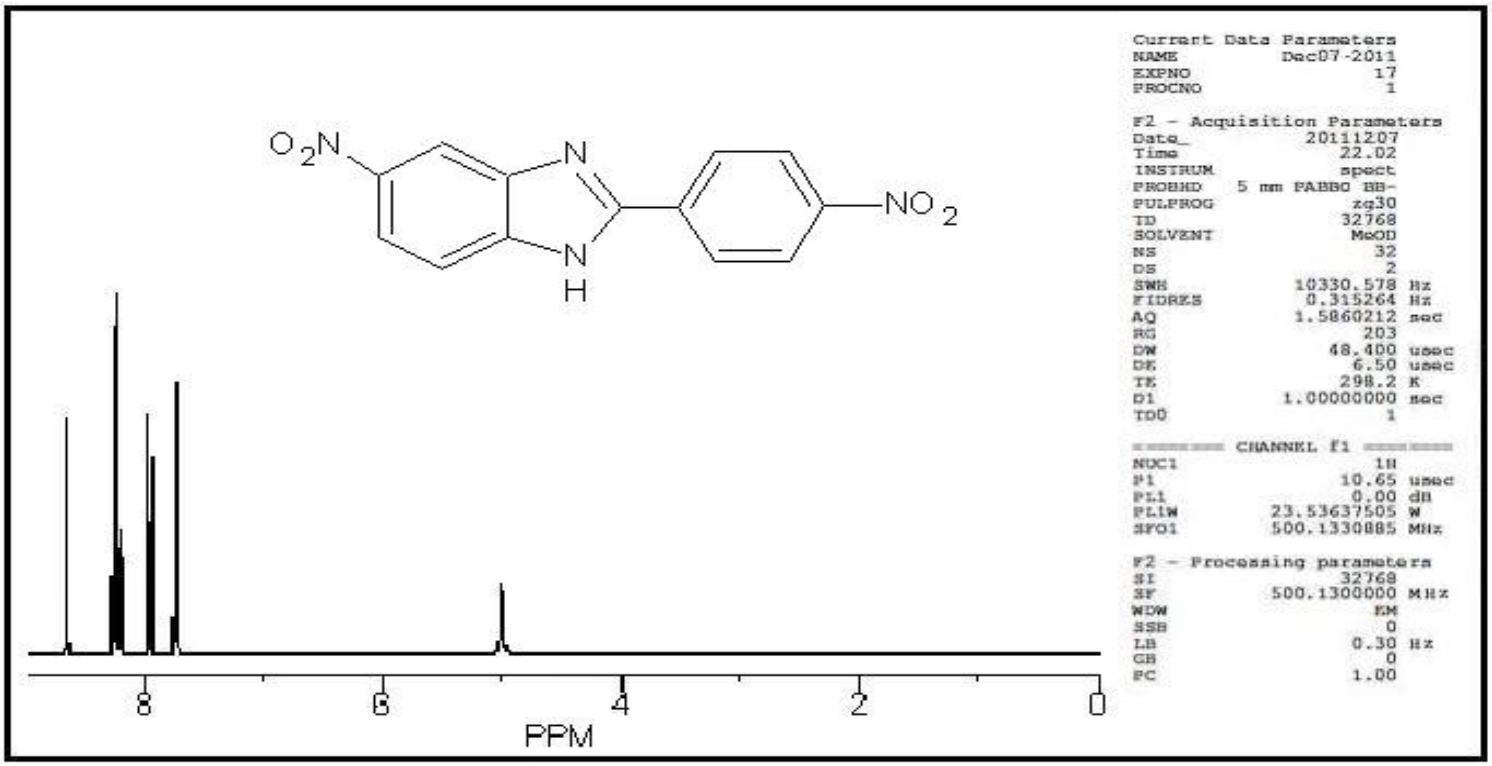


Fig-56: ^{1}H-NMR Spectra of the compound SY_{10}

6.2.11. Spectral analysis of (5-amino-1*H*-benzimidazol-2-yl) methane thiol:

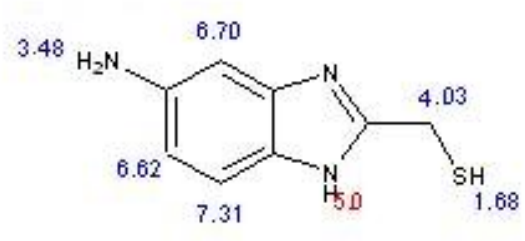
UV: (**Fig-58)**

λ_{max} (DMF, MeOH)	275.0 (ε_{max} 0.1927)

IR (KBr): (**Fig-59)**

Wave Number (Cm^{-1})	**Assignment**
3552.00	N-H asymmetrical stretching (primary amine)
3416.86	N-H symmetrical stretching (primary amine)
3376.18	N-H stretching (secondary amine)
2852.0	C-H stretching
2468.00	S-H stretching
1622.56	N-H bending
1448.97	C-H bending
1394.26	C-C ring stretching
1225.49	C-N stretching
1065.25	C-H bending
746.41	C-S stretching

NMR (DMSO – d_6): (Fig-60)



(3 aromatic protons, 2 aliphatic protons, 3 protons on nitrogen and 1 proton on sulphur)

δ	**Assignment**
7.31	(1H, d, Ar-H- C_7)
6.70	(1H, s, Ar-H- C_4)
6.62	(1H, d, Ar-H- C_6)
5.0	(1H, s, NH)
4.03	(2H, s, CH_2)
3.48	(2H, s, NH_2)
1.68	(1H, s, SH)

MASS: (Fig-61)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

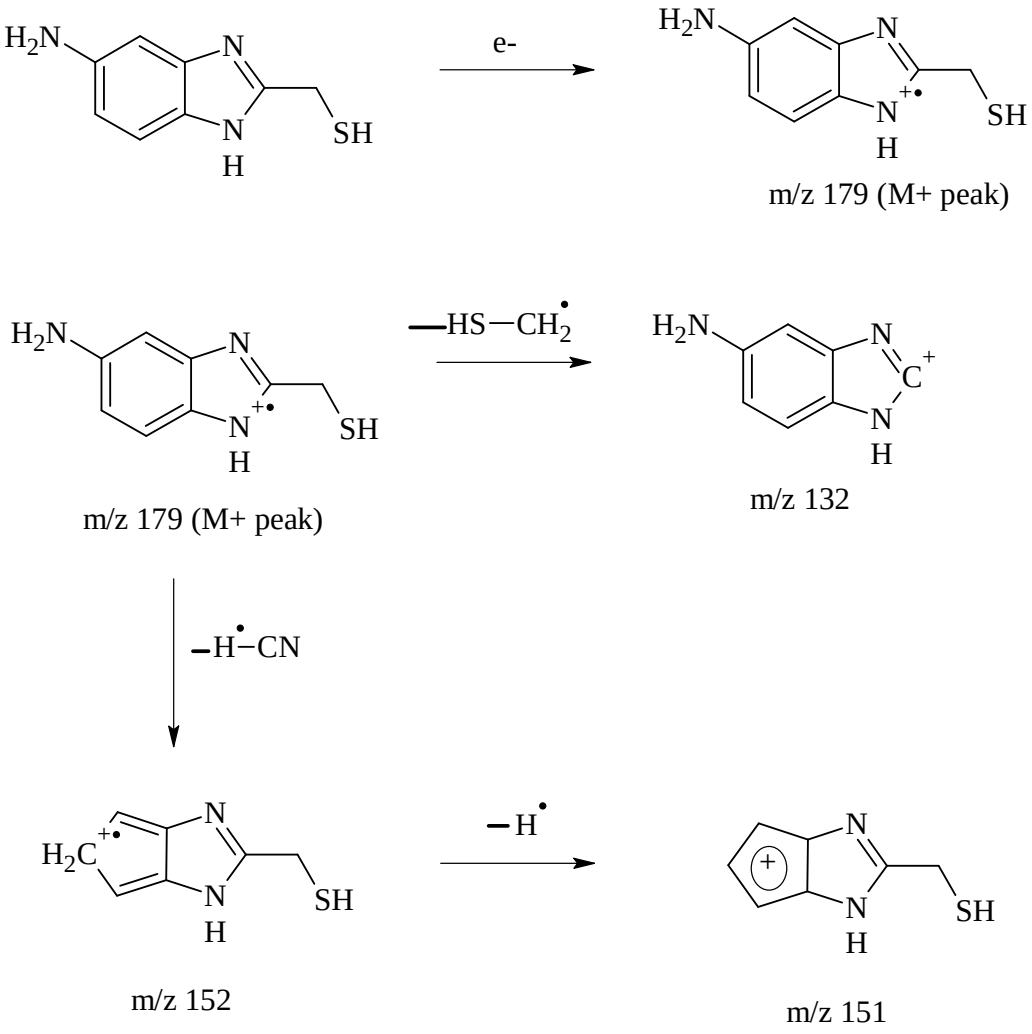


Fig-57: Fragmentation pattern of (5-amino-1*H*-benzimidazol-2-yl) methane thiol

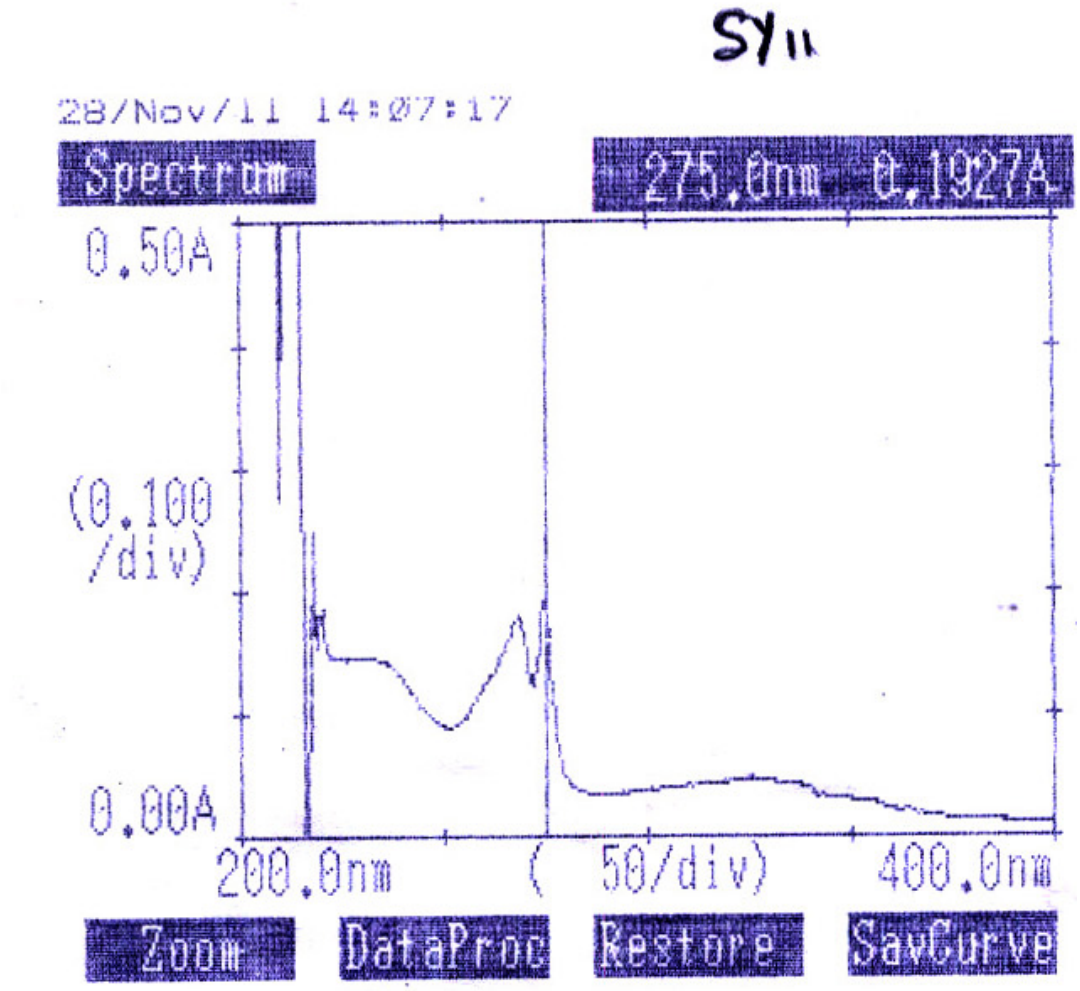


Fig-58: UV Spectrum of compound SY_{11}

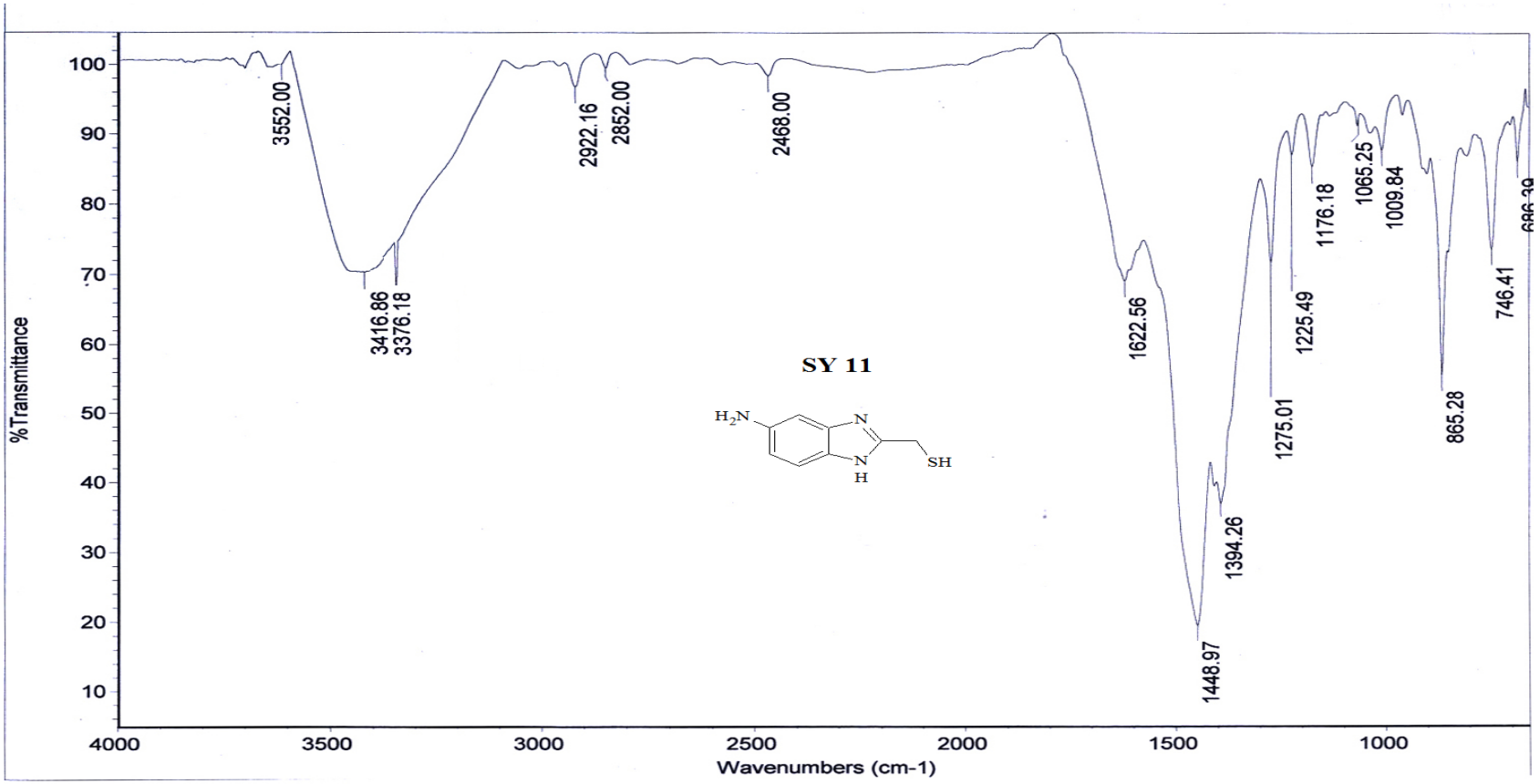


Fig-59: IR Spectrum of compound SY_{11}

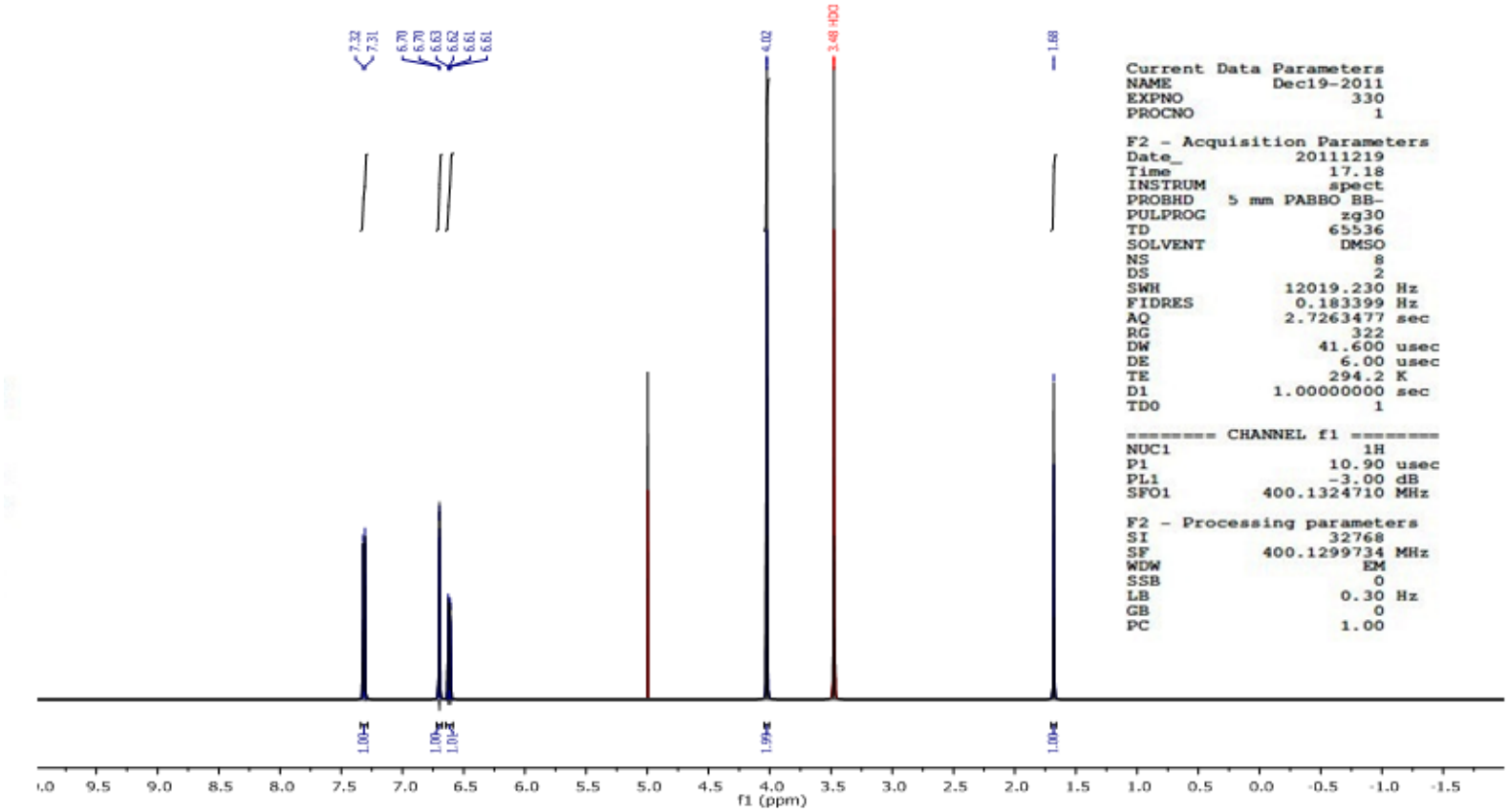


Fig-60: ^{1}H-NMR Spectrum of the compound SY_{11}

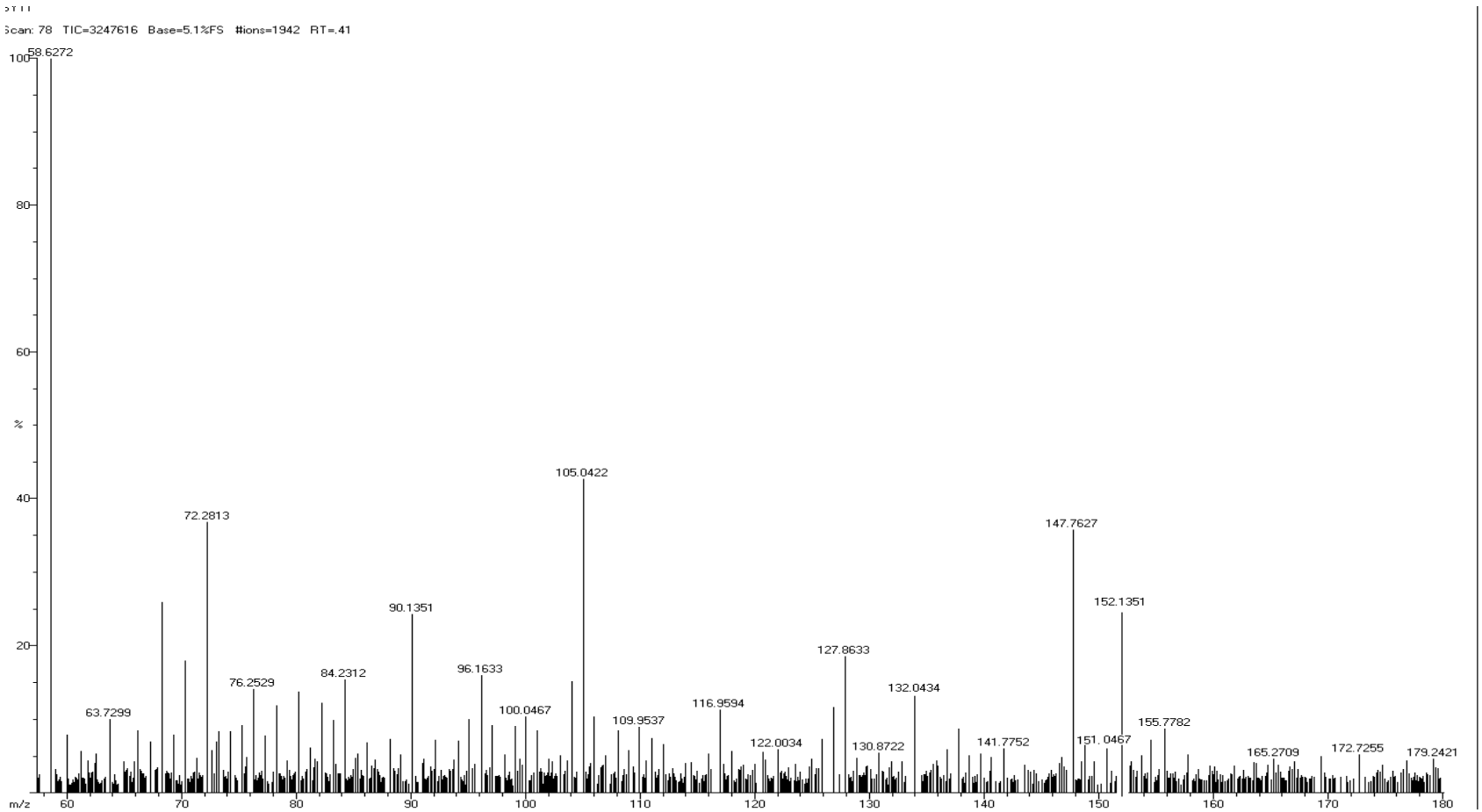


Fig-61: MASS Spectrum of the compound SY_{11}

6.2.12. Spectral analysis of 5-amino 2-(propan-2-yl)-1*H*-benzimidazole:

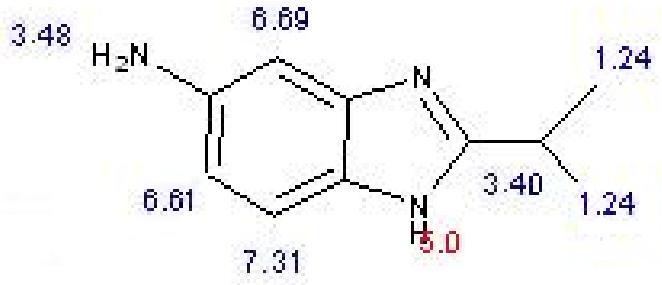
UV: (**Fig-63)**

λ_{max} (DMF, MeOH)	272.5 (ε_{max} 0.3374)

IR (KBr): (**Fig-64)**

Wave Number (Cm^{-1})	**Assignment**
3587.00	N-H asymmetrical stretching (primary amine)
3218.00	N-H stretching (secondary amine)
2343.00	C-H stretching
1635.00	N-H bending
1385.00	C-N stretching
1129.00	C-H in plane bending of aromatics

NMR (DMSO – d_6): (**Fig-65)**



(3 aromatic protons, 7 aliphatic protons, and 3 protons on nitrogen)

δ	**Assignment**
7.31	(1H, d, Ar-H- C_7)
6.69	(1H, s, Ar-H- C_4)
6.61	(1H, d, Ar-H- C_6)
5.0	(1H, s, NH)
3.48	(2H, s, NH_2)
3.40	(1H, m, CH of isopropyl)
1.24	(6H, m, CH_3 of isopropyl)

MASS: (Fig-66)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

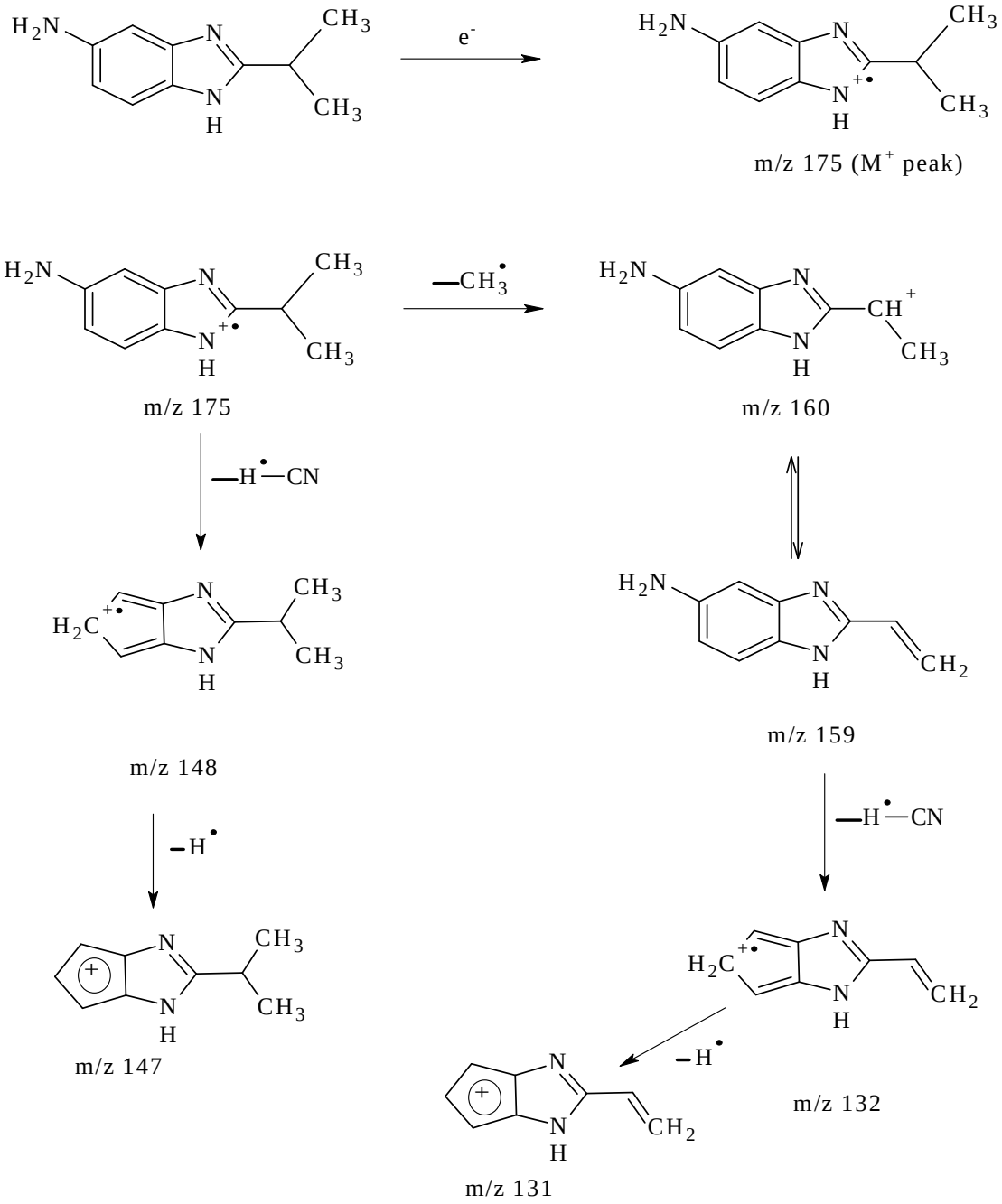
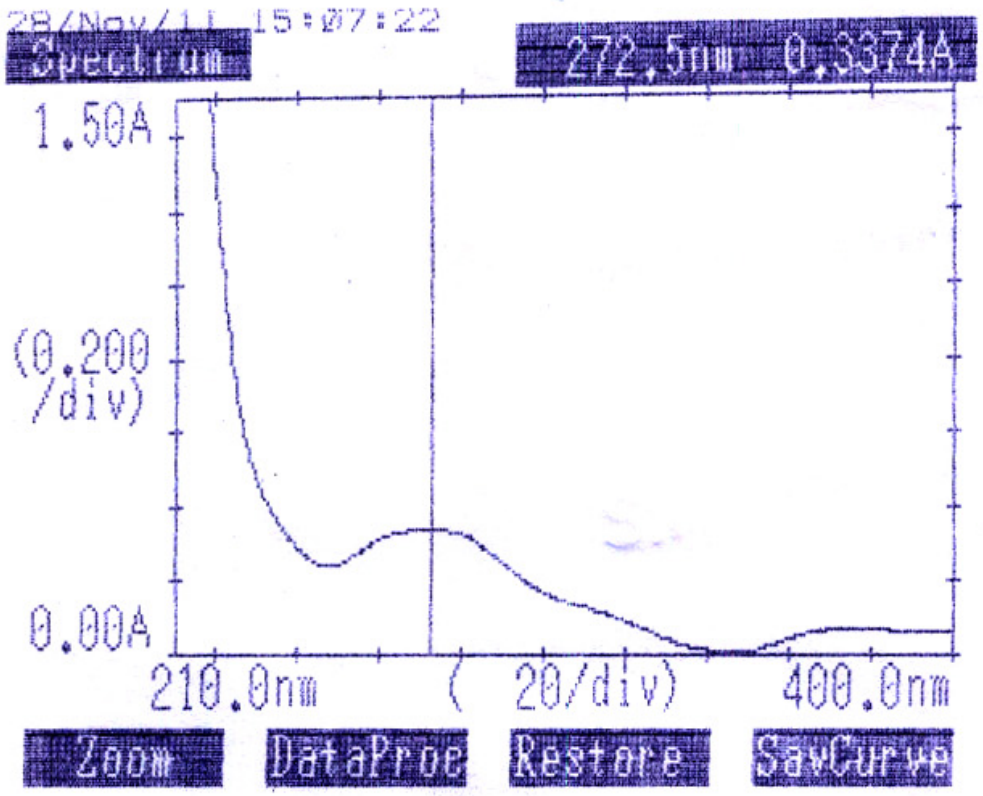


Fig-62: Fragmentation pattern of 5-amino 2-(propan-2-yl)-1*H*-benzimidazole



(Fig-63: UV Spectrum of compound SY_{12})

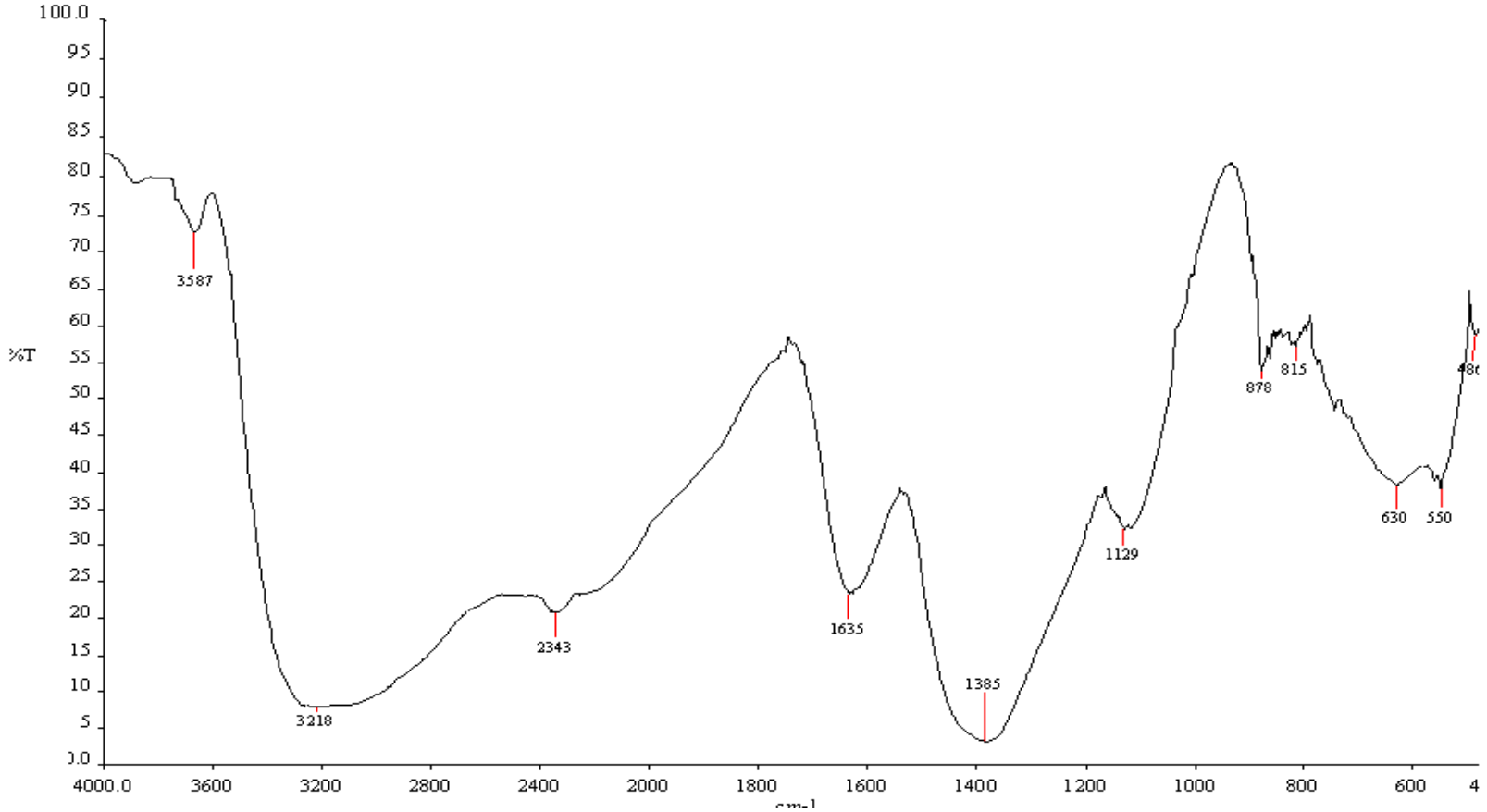


Fig-64: IR Spectrum of compound SY_{12}

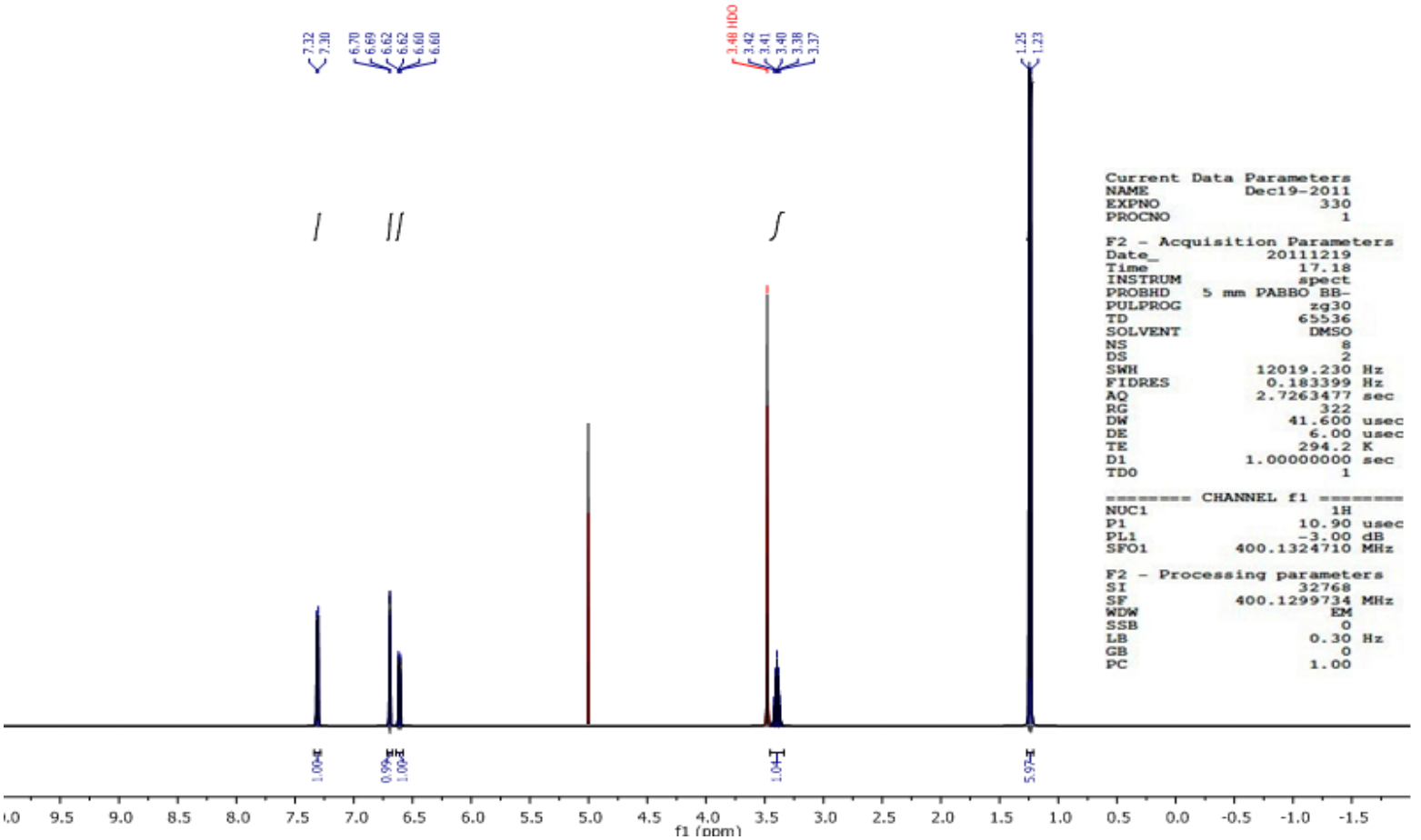


Fig 65: ^{1}H-NMR Spectrum of the compound SY_{12}

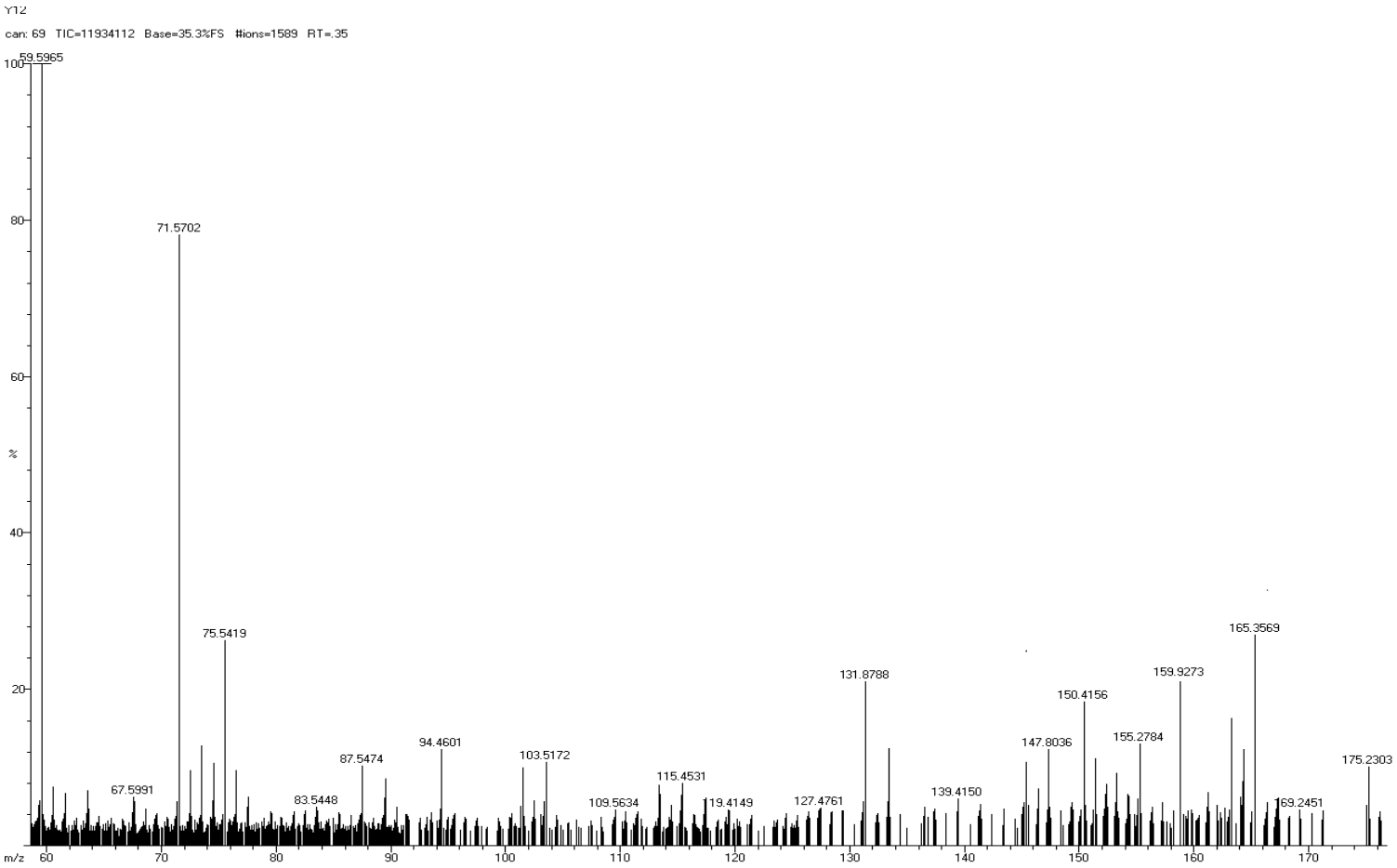


Fig-66: MASS Spectrum of the compound SY_{12}

6.2.13. Spectral analysis of 5-amino 2-butyl-1*H*-benzimidazole:

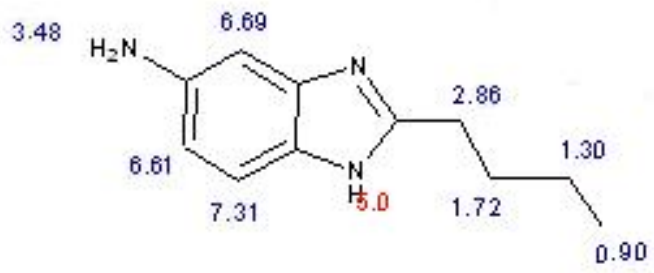
UV: (Fig-68)

λ_{max} (DMF, MeOH)	366.0 (ε_{max} 0.1759)

IR (KBr): (Fig-69)

Wave Number (Cm^{-1})	**Assignment**
3573.00	N-H asymmetrical stretching (primary amines)
3393.00	N-H stretching (secondary amines)
2175.00	C-H stretching
1648.00	N-H bending
1397.00	C-N stretching
1113.00	C-H bending (wagging)
689.00	C-H bending (rocking)

NMR (DMSO – d_6): (Fig-70)



(3 aromatic protons, 9 aliphatic protons, and 3 protons on nitrogen)

δ	**Assignment**
7.31	(1H, d, Ar-H- C_7)
6.69	(1H, s, Ar-H- C_4)
6.61	(1H, d, Ar-H- C_6)
5.0	(1H, s, NH)
3.48	(2H, s, NH_2)
2.86	(2H, t, CH_2)
1.72	(2H, pent, CH_2)
1.30	(2H, m, CH_2)
0.90	(3H, t, CH_3)

MASS: (Fig-71)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

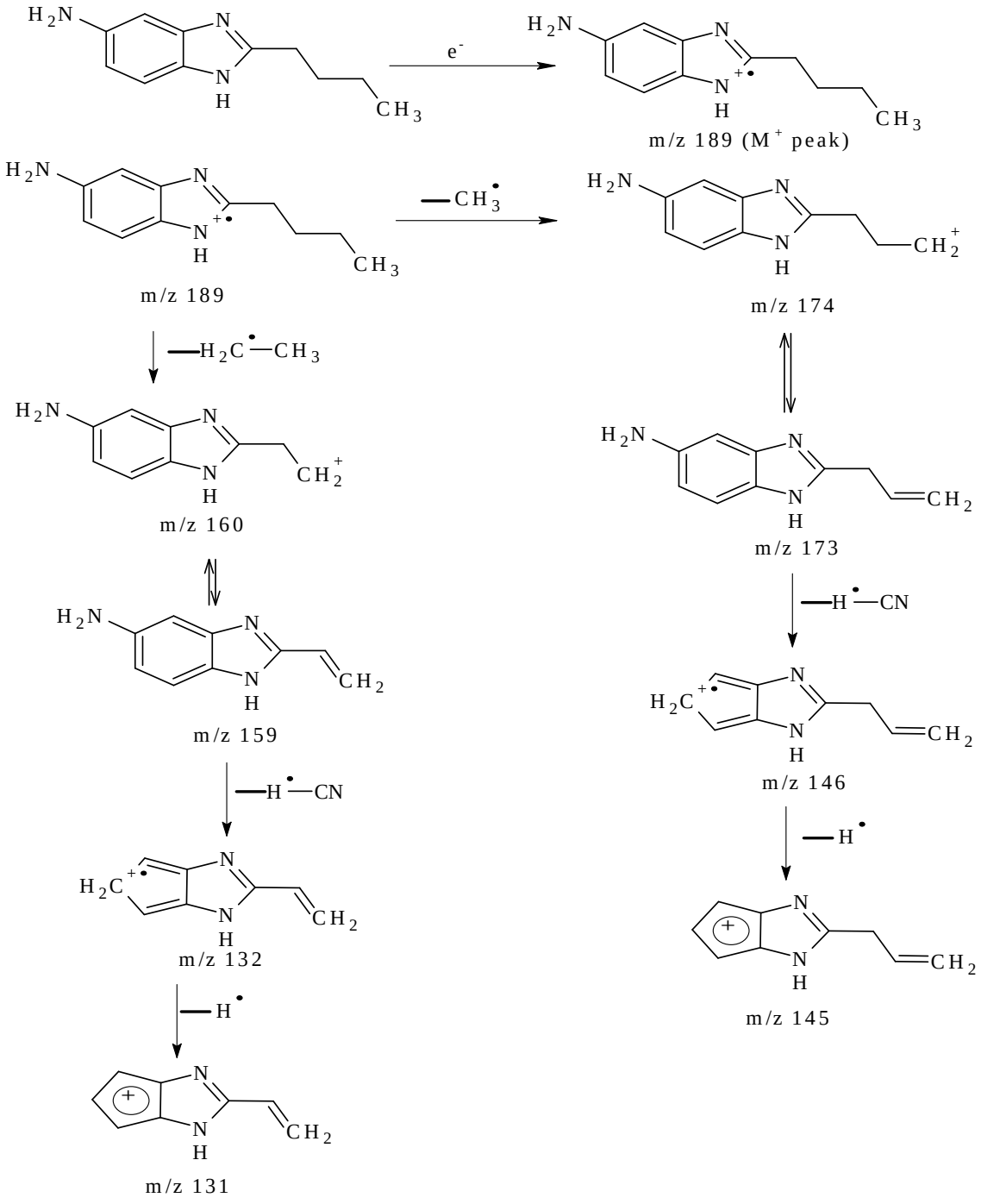
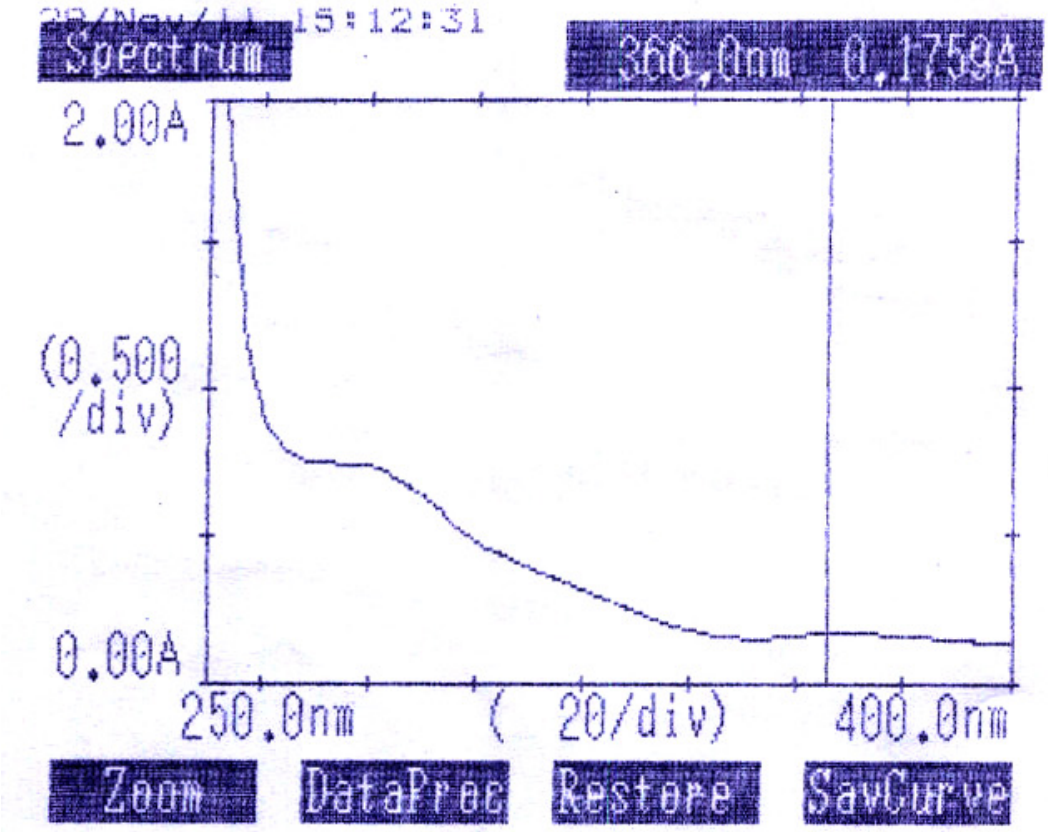


Fig-67: Fragmentation pattern of 5-amino 2-butyl-1*H*-benzimidazole



(Fig-68: UV Spectrum of compound SY_{13})

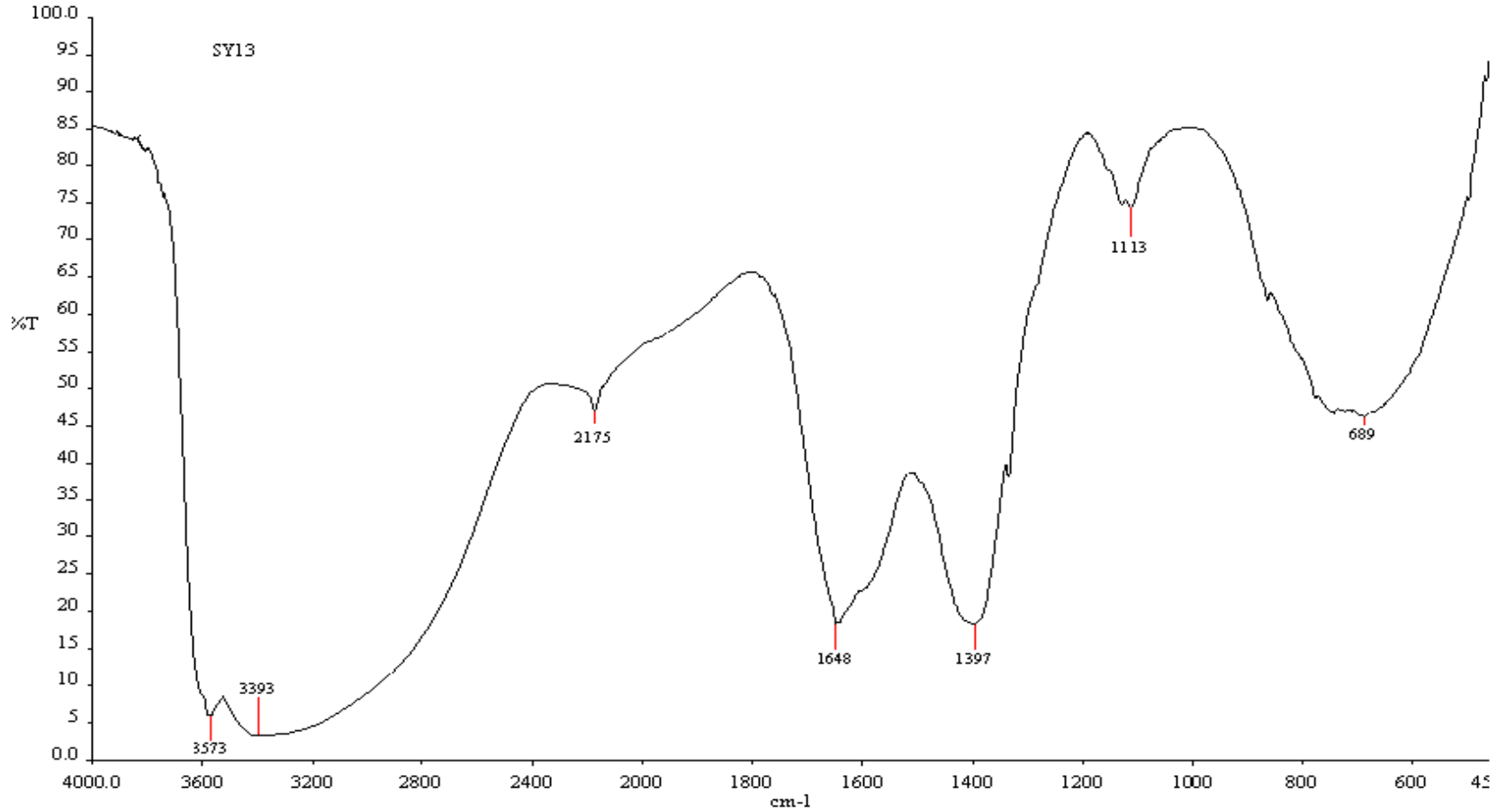


Fig-69: IR Spectrum of compound SY_{13}

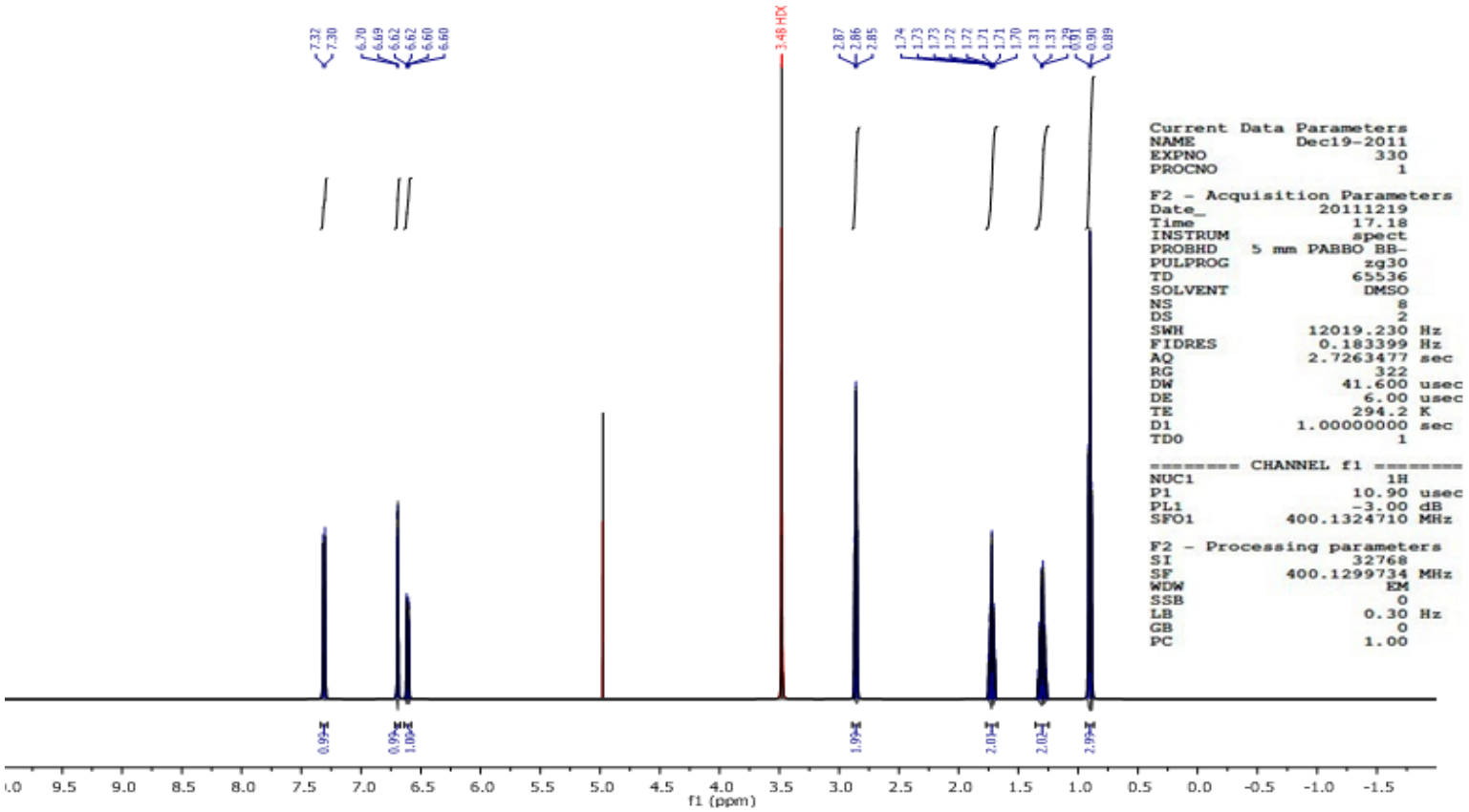


Fig-70: ^{1}H-NMR Spectrum of the compound SY_{13}

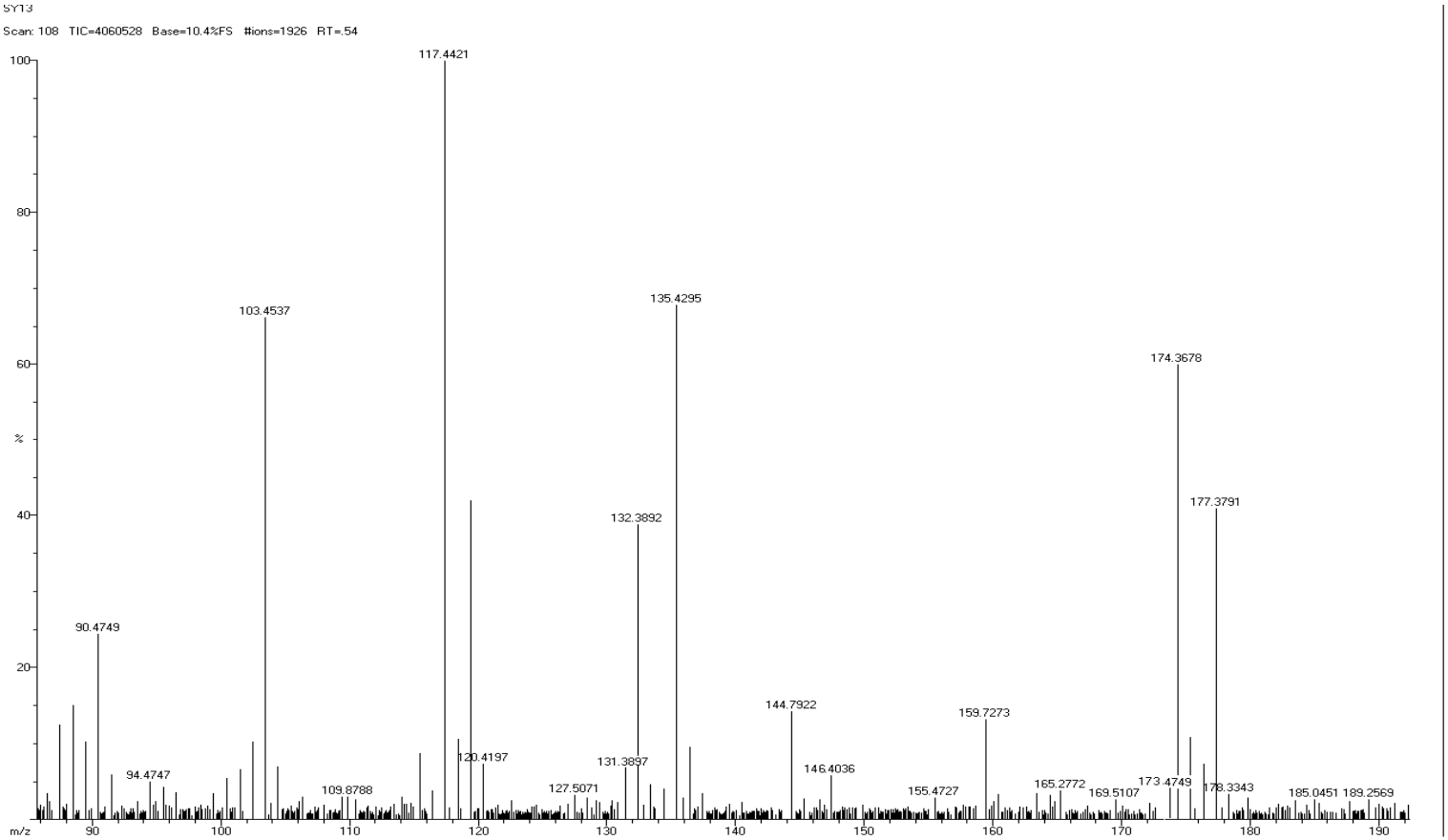


Fig-71: MASS Spectrum of the compound SY_{13}

6.2.14. Spectral analysis of 5amino 2-(4-aminophenyl)-1*H*-benzimidazole:

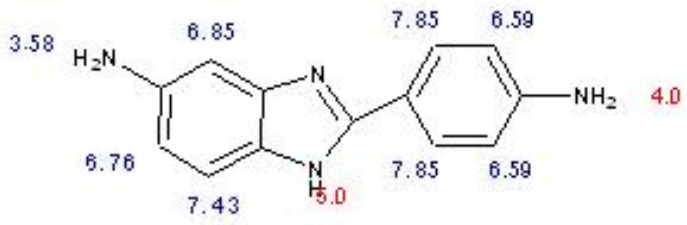
UV: (**Fig-73)**

λ_{max} (DMF, MeOH)	309.5 (ε_{max} 0.2688)

IR (KBr): (Fig-74)

Wave Number (Cm^{-1})	**Assignment**
3579.00	N-H asymmetrical stretching (primary amine)
3369.00	N-H stretching (secondary amine)
1645.00	N-H bending
1429.00	C-N stretching
866.00	C-H bending
657.00	C-H bending of aromatics

NMR (DMSO – d_6): (Fig-75)



(7 aromatic protons and 5 protons on nitrogen)

δ	Assignment
7.85	(2H, m, Ar-H- $C_{2'}$&$C_{6'}$)
7.43	(1H, d, Ar-H- C_7)
6.85	(1H, s, Ar-H- C_4)
6.76	(1H, d, Ar-H- C_6)
6.59	(2H, m, Ar-H- C_{10}&C_{12})
5.0	(1H, s, NH)
3.99	(2H, s, NH_2)
3.58	(2H, s, NH_2)

MASS: (Fig-76)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

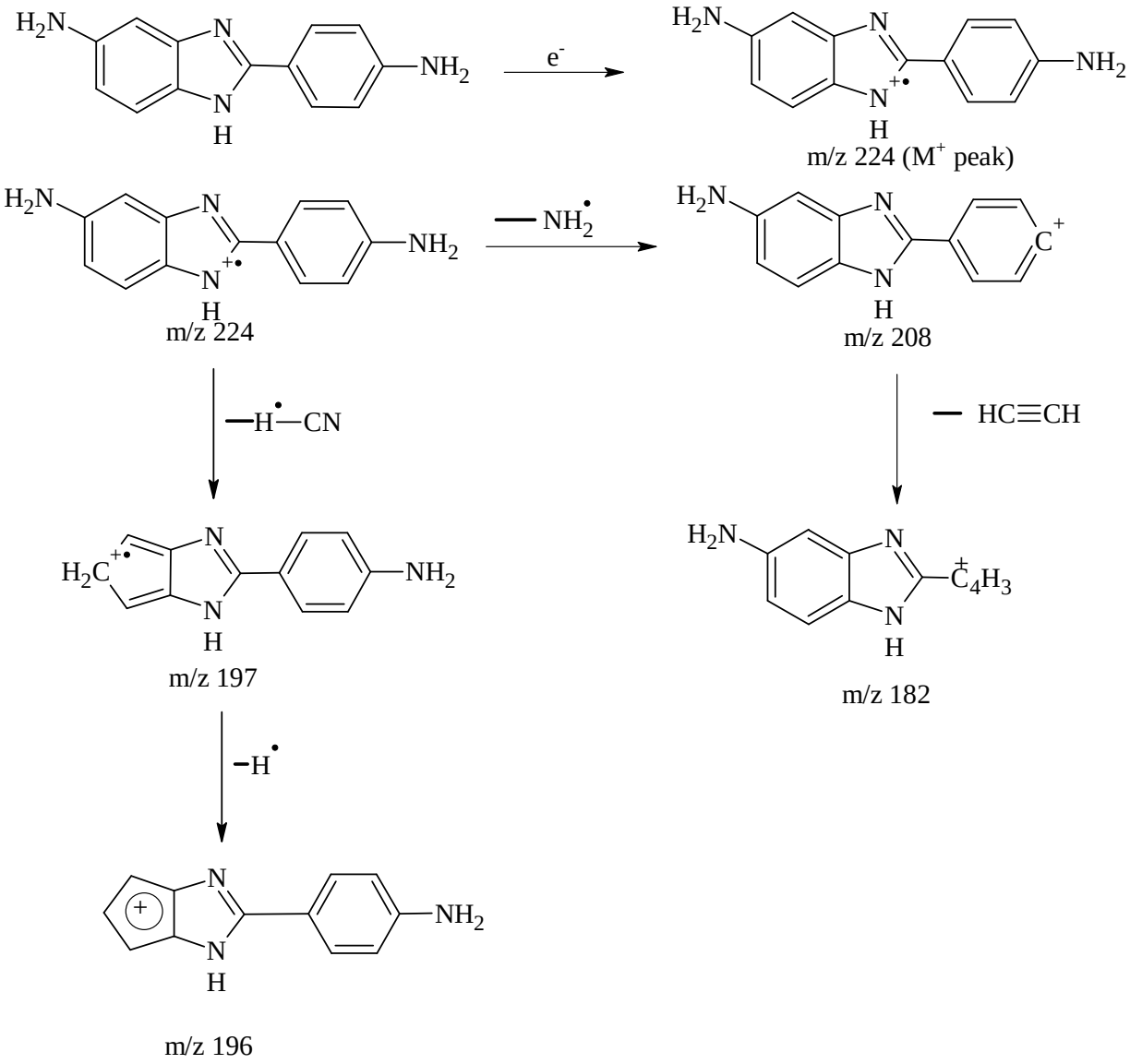


Fig-72: Fragmentation pattern of 5 amino 2-(4-aminophenyl)-1*H*-benzimidazole

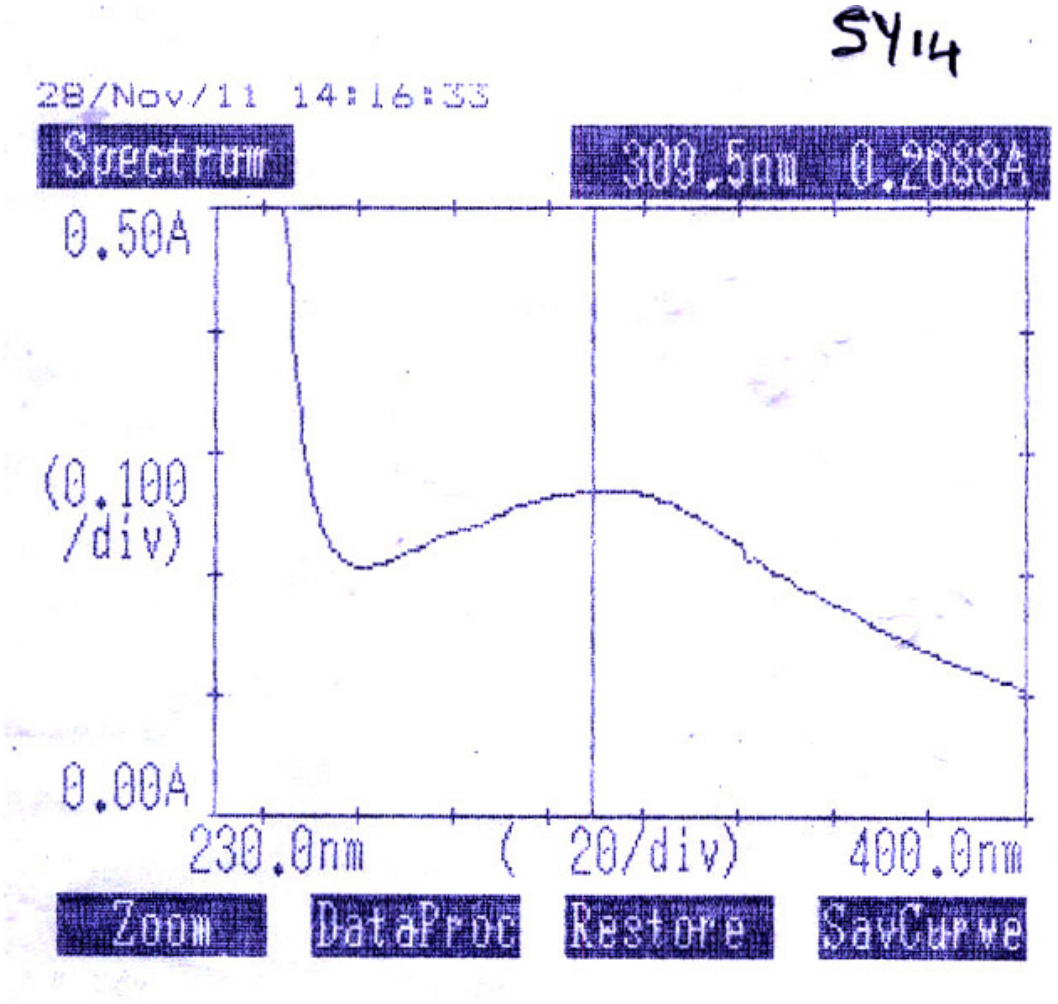


Fig-73: UV Spectrum of compound SY_{14}

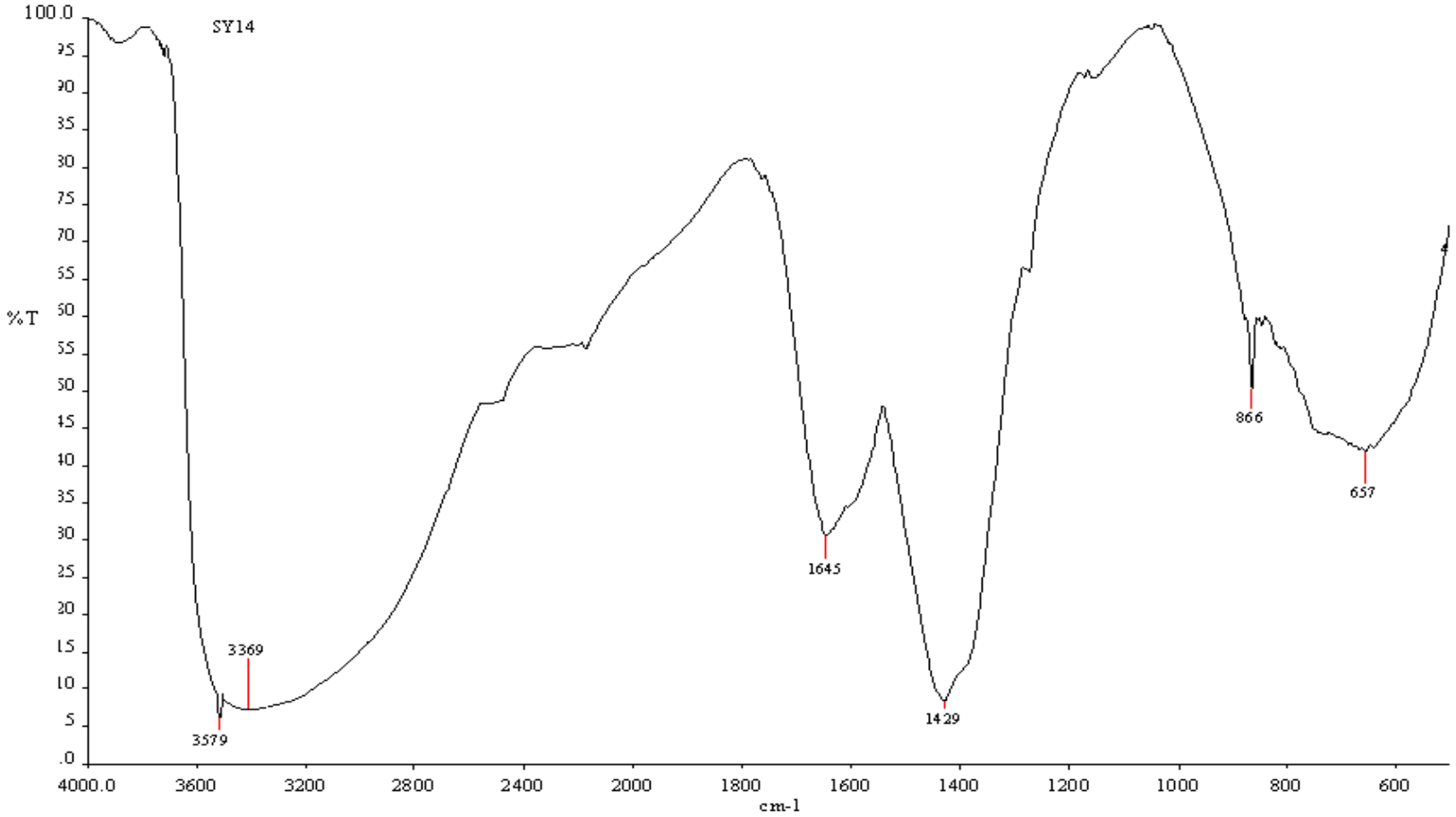


Fig-74: IR Spectrum of compound SY_{14}

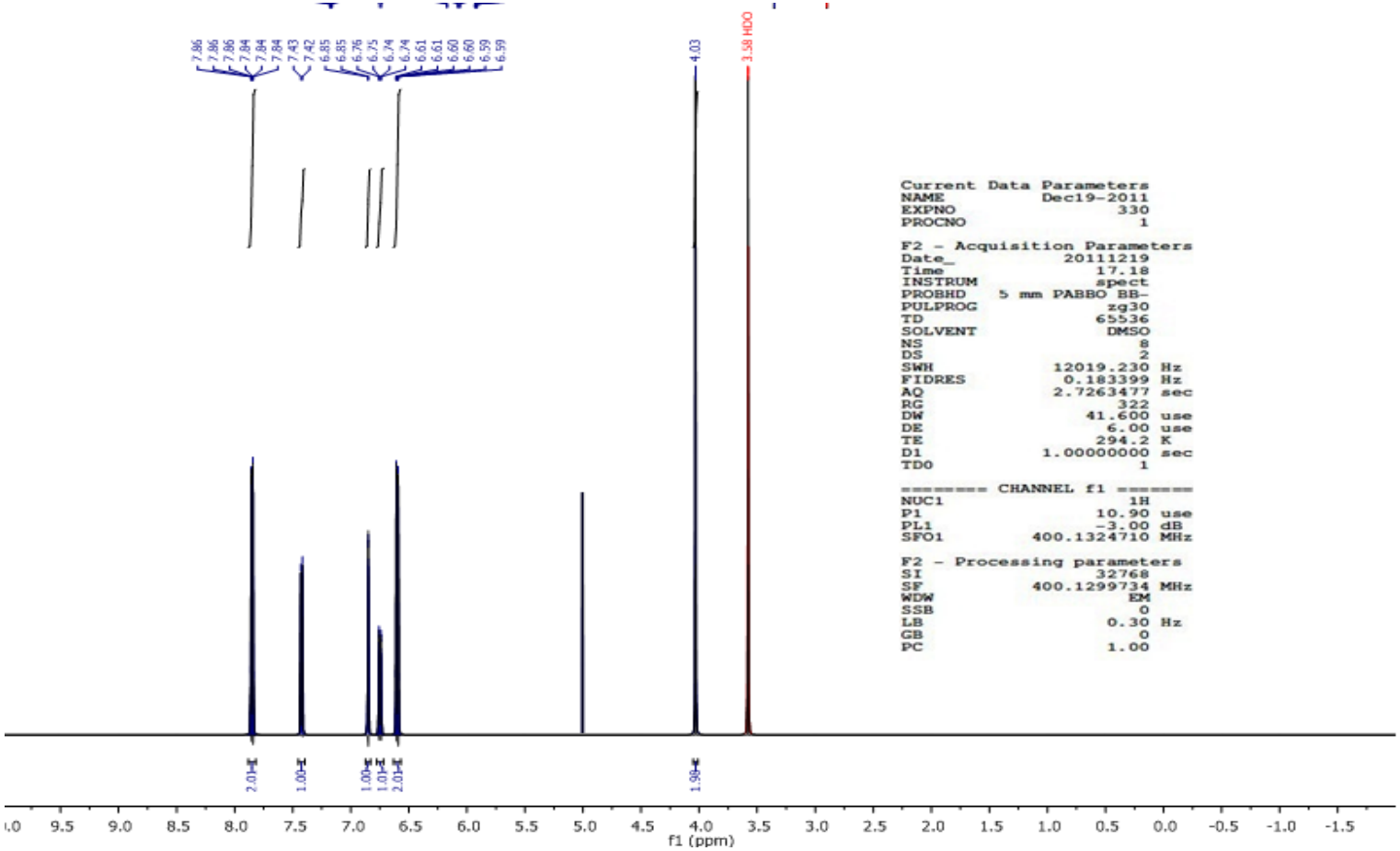


Fig-75: ^{1}H-NMR Spectrum of the compound SY_{14}

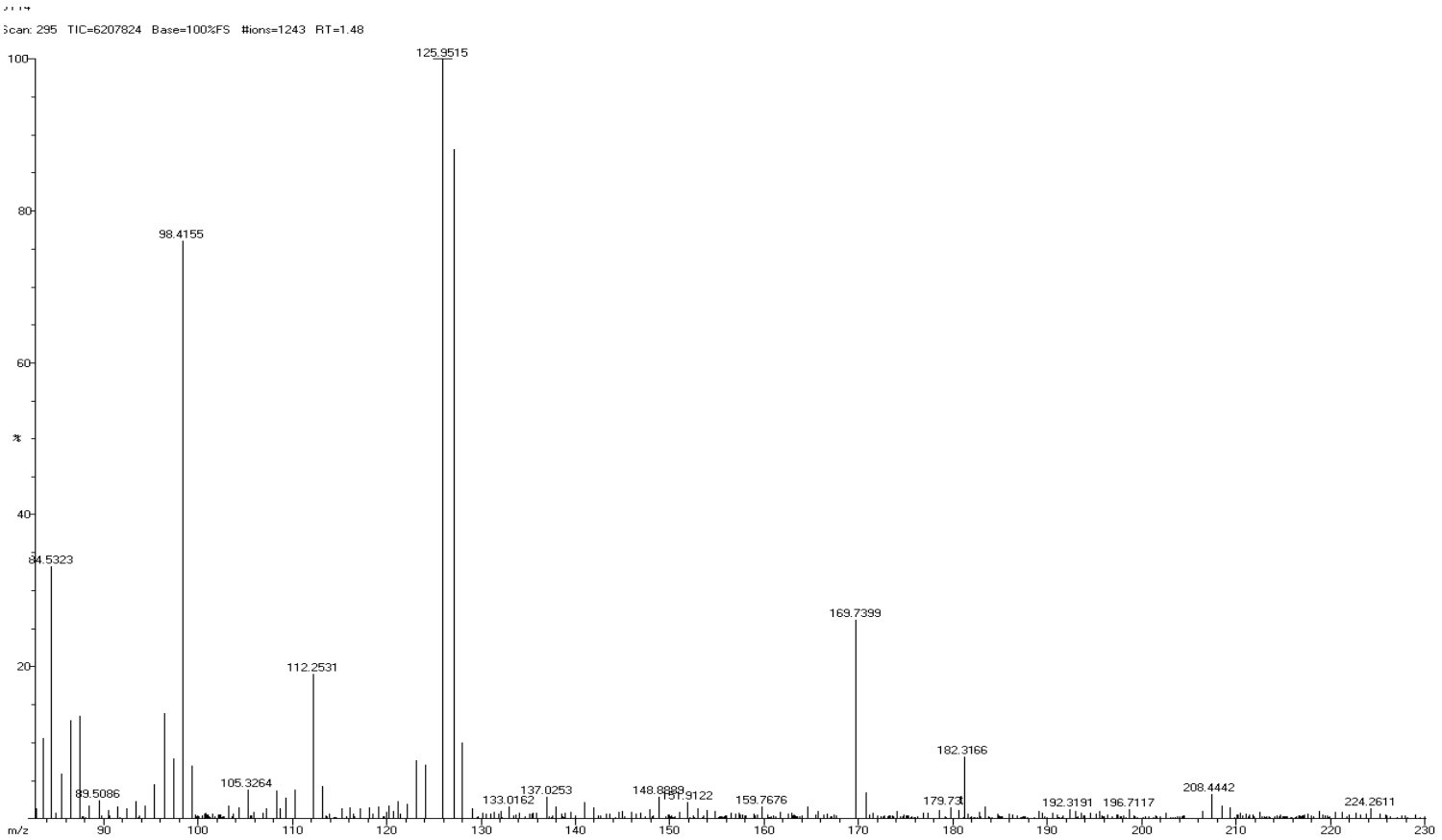


Fig-76: MASS Spectrum of the compound SY_{14}

6.2.15. Spectral analysis of 5-amino 2-(4-nitrophenyl)-1*H*-benzimidazole:

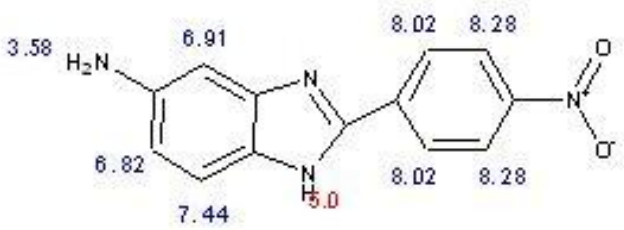
UV: (Fig-78)

λ_{max} (DMF, MeOH)	270.0 (ε_{max} 0.0392)

IR (KBr): (Fig-79)

Wave Number (Cm^{-1})	**Assignment**
3559.00	N-H asymmetrical stretching (primary amine)
3432.00	N-H symmetrical stretching (primary amine)
3206.00	N-H stretching (secondary amine)
1632.00	N-H bending
1596.00	N-O stretching
864.00	Out of plane bending of aromatics
795.00	C-H bending

NMR (DMSO – d_6): (Fig-80)



(7 aromatic protons and 3 protons on nitrogen)

δ	**Assignment**
8.28	(2H, m, Ar-H- $C_{3'}$&$C_{5'}$)
8.02	(2H, m, Ar-H- $C_{2'}$&$C_{6'}$)
7. 44	(1H, d, Ar-H- C_7)
6.91	(1H, s, Ar-H- C_4)
6.82	(1H, d, Ar-H- C_6)
5.0	(1H, s, NH)
3.58	(2H, s, NH_2)

MASS: (Fig-81)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

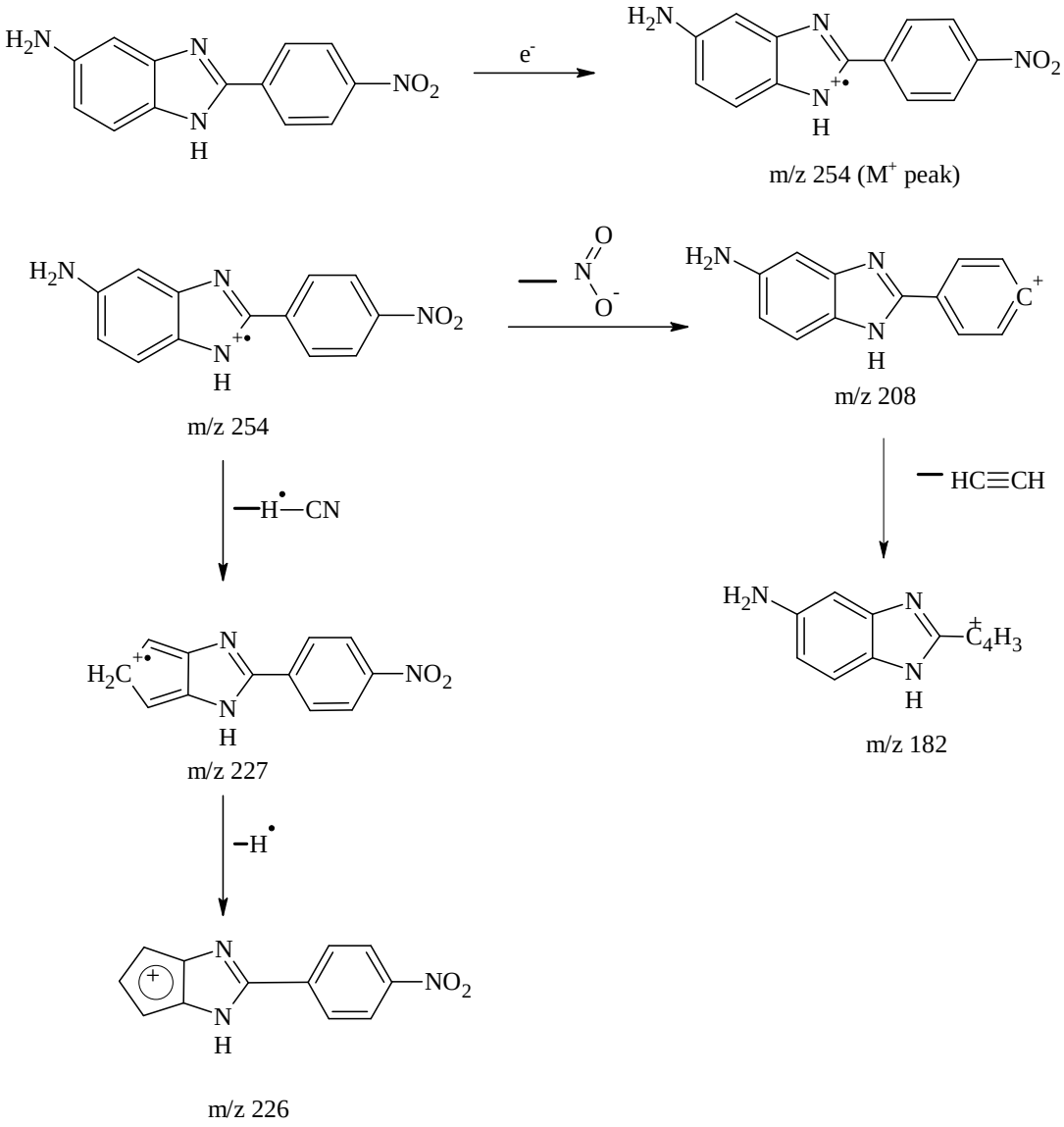


Fig-77: Fragmentation pattern of 5-amino 2-(4-nitrophenyl)-1*H*-benzimidazole

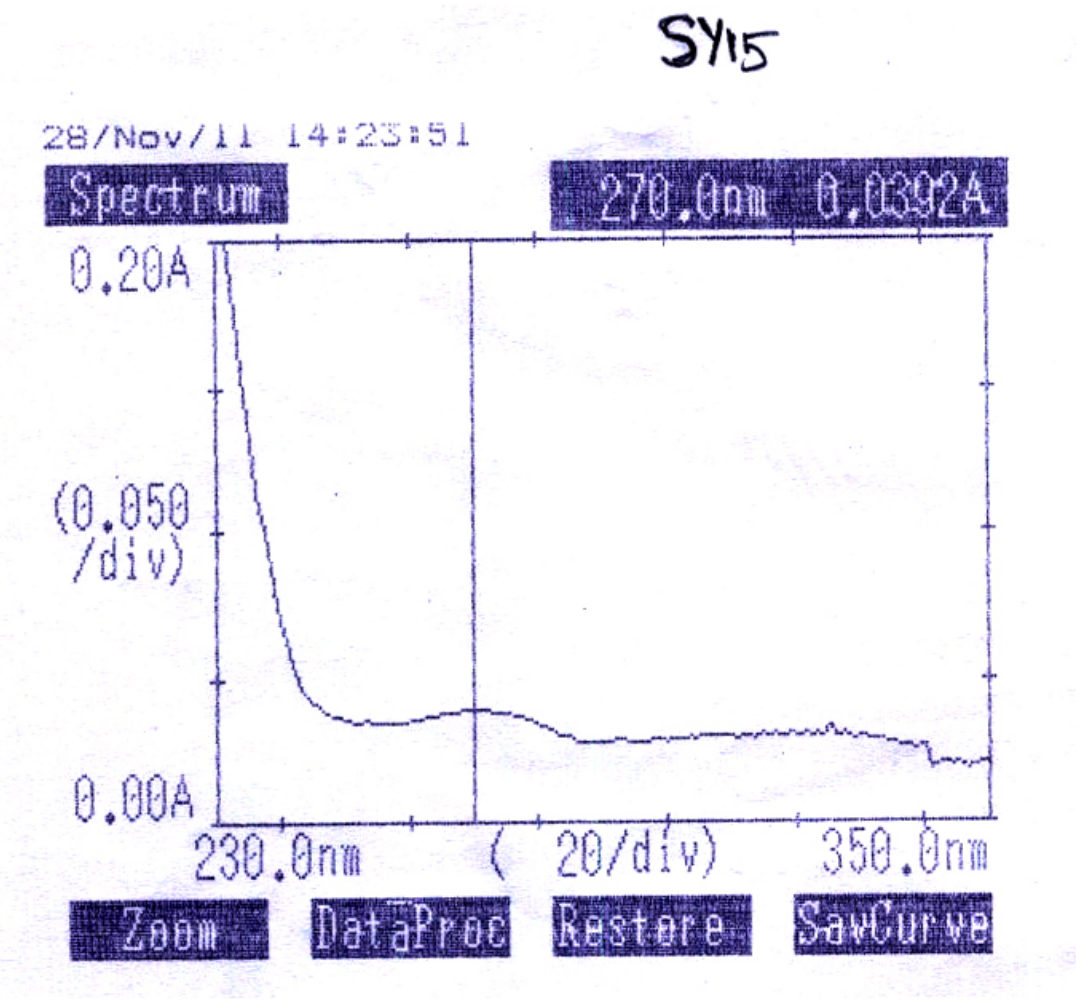


Fig-78: UV Spectrum of compound SY_{15}

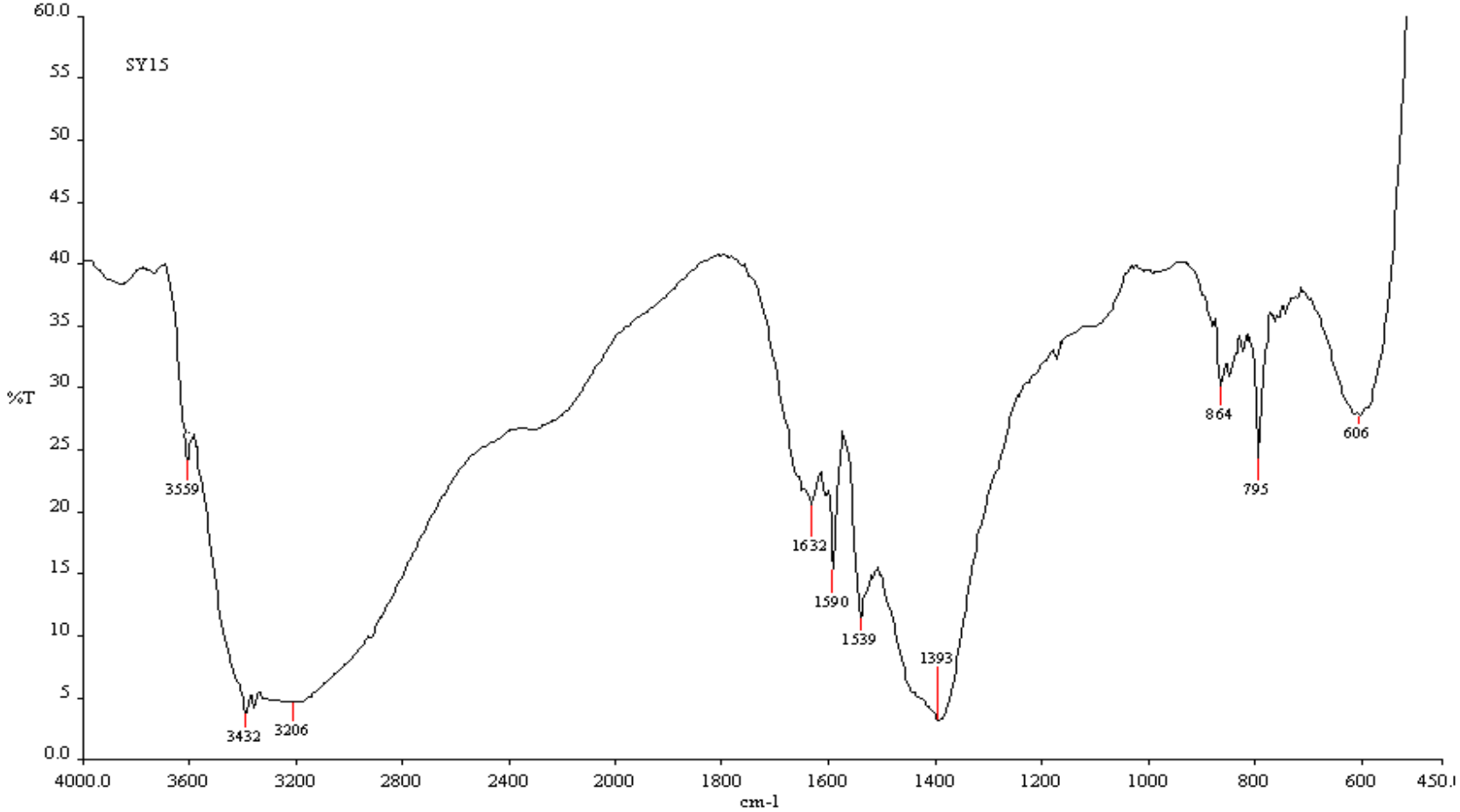


Fig-79: IR Spectrum of compound SY_{15}

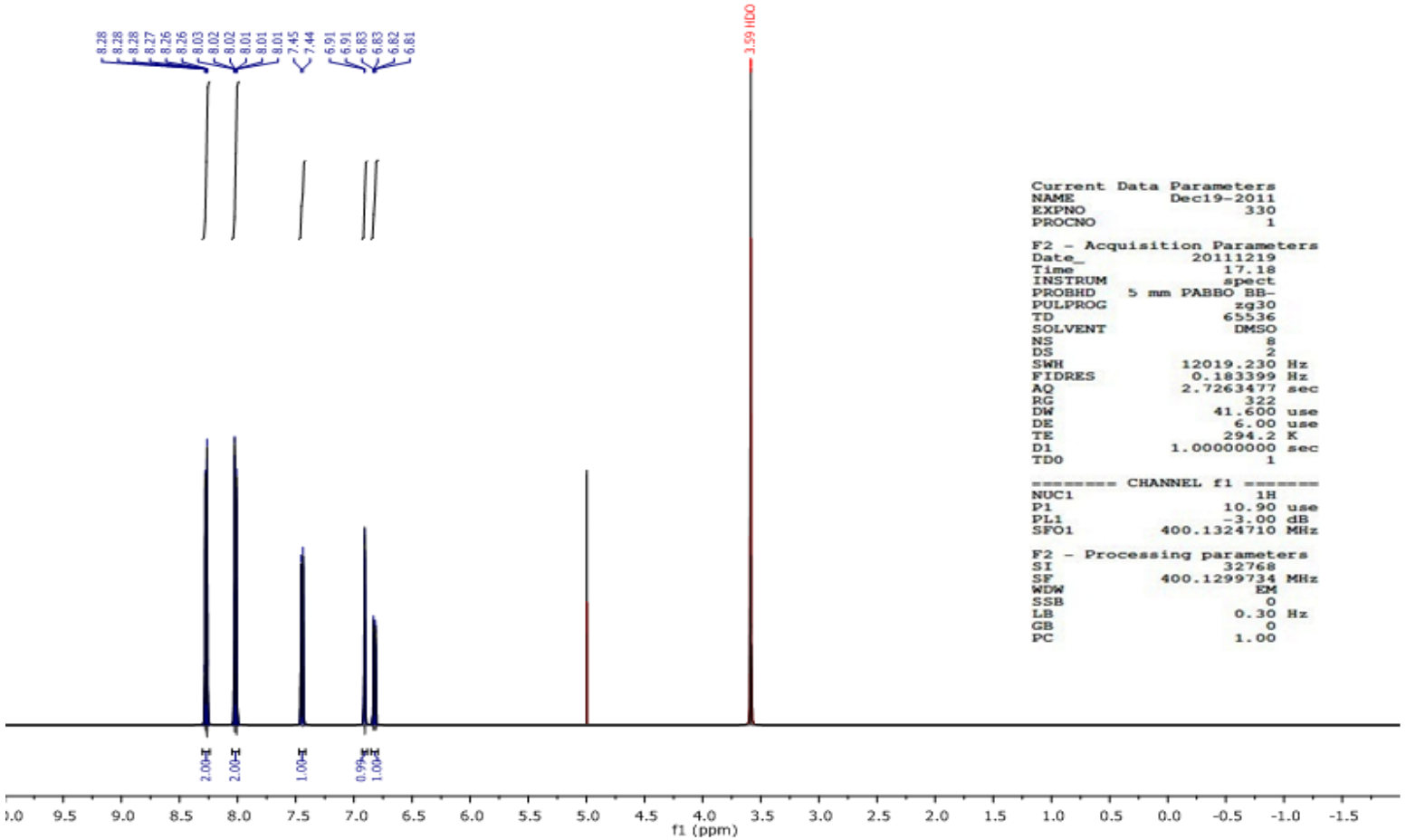


Fig-80: ^{1}H-NMR Spectrum of the Compound SY_{15}

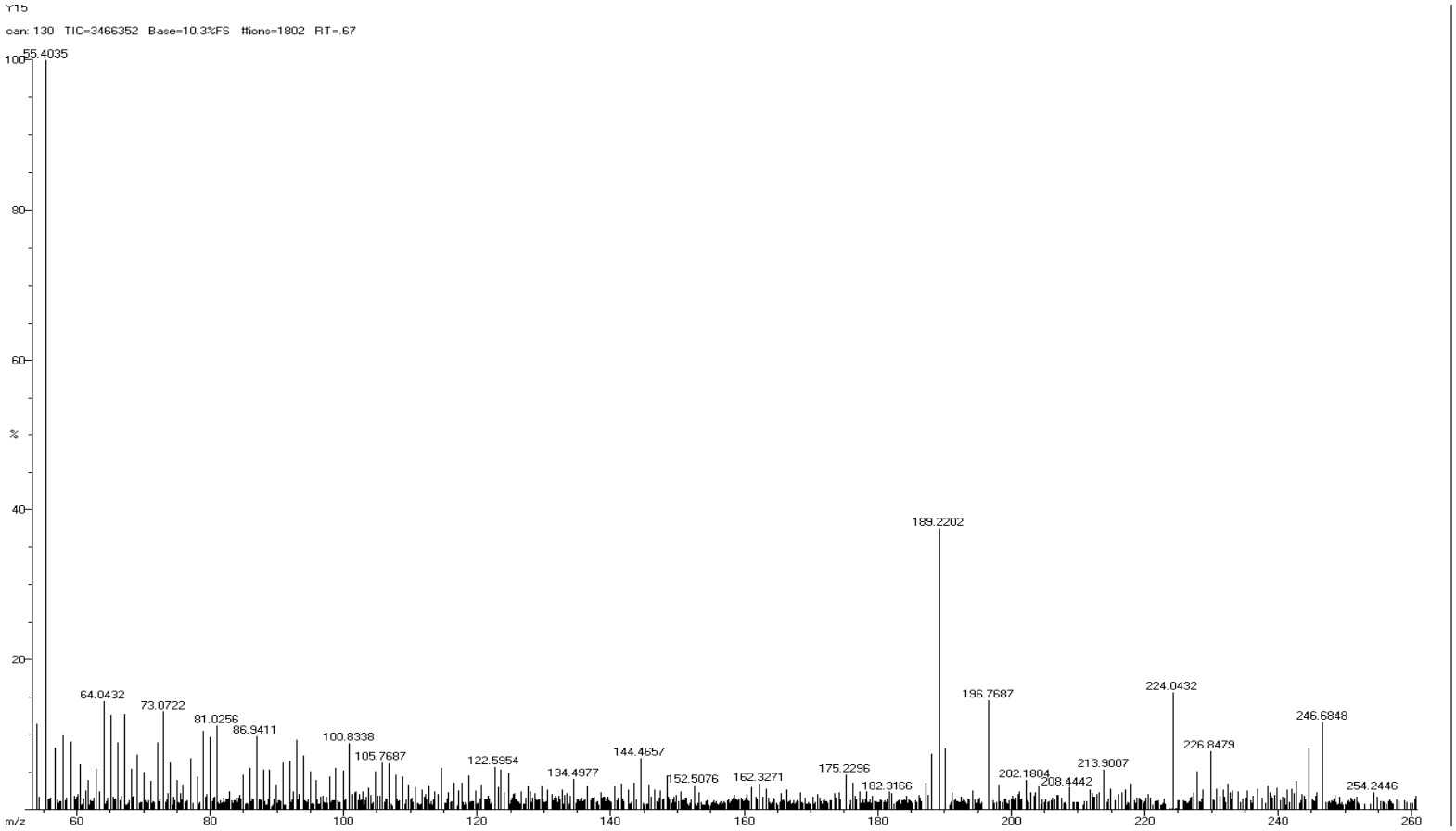


Fig-81: MASS Spectrum of the compound SY_{15}

Table-1: Physical and analytical data of the synthesized compounds

Code	Name	Nature	Solubility	Molecular weight (gm)	Molecular formula	Melting Point (^{O}C)	Percentage Yield (%)	R_f Value Methanol : water (8:2)
SY_1	1*H*-benzimidazol-2-yl methane thiol	Dark green colour powder	Freely soluble: Methanol, ethyl acetate, ethanol and acetone Sparingly soluble: water, benzene and chloroform	164.22	$C_8H_8N_2S$	162	37.94	0.79
SY_2	2-(propan-2-yl)-1*H*-benzimidazole	Dark brown colour crystal	Freely soluble: Methanol, ethanol, ethyl acetate, benzene, chloroform and acetone Sparingly soluble: water	160.21	$C_{10}H_{12}N_2$	112	39.94	0.85

SY_3	2-butyl-1*H*-benzimidazole	Light brown colour crystals	Freely soluble: Methanol, ethyl acetate, ethanol and acetone Sparingly soluble: benzene, chloroform and water	174.24	$C_{11}H_{14}N_2$	114	54.02	0.83
SY_4	4-(1*H*-benzimidazol-2-yl) aniline	Pink colour solid	Freely soluble: Methanol, ethyl acetate, benzene, chloroform and acetone Soluble: ethanol Sparingly soluble: hexane and water	209.24	$C_{13}H_{11}N_3$	248 (248-250)	35.37	0.30
SY_5	2-(4-nitrophenyl) -1*H*-benzimidazole	White colour powder	Freely Soluble: Methanol and acetone Sparingly soluble: ethanol, ethyl acetate, hexane and water Very slightly soluble: benzene and chloroform	239.23	$C_{13}H_9N_3O_2$	254 (254-256)	31.38	0.58

SY_6	(5-nitro-1*H*-benzimidazol-2-yl) methane thiol	Light yellow crystalline powder	Soluble: Methanol and acetone Sparingly soluble: ethyl acetate, chloroform and ethanol	209.22	$C_8H_7N_3O_2S$	185	54.79	0.81
SY_7	5-nitro 2-(propan-2-yl)-1*H*-benzimidazole	Dark black colour solid	Freely soluble: Methanol Sparingly soluble: ethyl acetate, acetone and ethanol Slightly soluble: benzene and chloroform	205.21	$C_{10}H_{11}N_3O_2$	96	31.53	0.69
SY_8	5-nitro 2-butyl-1*H*-benzimidazole	Dark black colour solid	Freely soluble: Methanol Sparingly soluble: ethanol and chloroform Slightly soluble: ethyl acetate and acetone	219.24	$C_{11}H_{13}N_3O_2$	124	26.59	0.84

SY_9	4-(5-nitro-1*H*-benzimidazol-2-yl) aniline	Light yellow crystalline powder	Soluble: Methanol and ethyl acetate Sparingly soluble: ethanol, acetone and chloroform	254.24	$C_{13}H_{10}N_4O_2$	216	30.09	0.81
SY_{10}	5-nitro 2-(4-nitrophenyl)-1*H*-benzimidazole	White colour powder	Sparingly soluble: Methanol and ethanol Slightly soluble: ethyl acetate and water	284.22	$C_{13}H_8N_40_4$	226	39.54	0.58
SY_{11}	(5-amino-1*H*-benzimidazol-2-yl) methane thiol	Light brown colour solid	Freely soluble: Dimethylformamide and Dimethylsufoxide Sparingly soluble: methanol Slightly soluble: chloroform and benzene Very slightly soluble: ethanol, acetone and water	179.24	$C_8H_9N_3S$	261	44.04	0.78

SY_{12}	5-amino 2-(propan-2-yl)-1*H*-benzimidazole	Black colour solid	Freely soluble: Dimethylformamide and Dimethylsufoxide Soluble: Methanol Sparingly soluble: Chloroform and water Slightly soluble: Benzene and hexane	175.23	$C_{10}H_{13}N_3$	220	91.76	0.79
SY_{13}	5-amino 2-butyl-1*H*-benzimidazole	Black colour solid	Freely soluble: Dimethylformamide and Dimethylsufoxide Soluble: Methanol Sparingly soluble: Chloroform and water Slightly soluble: benzene, hexane, ethanol	189.25	$C_{11}H_{15}N_3$	238	93.02	0.80

SY_{14}	5-amino (4-aminophenyl)-1*H*-benzimidazole	Dark black colour solid	Freely soluble: Dimethylformamide and Dimethylsufoxide Sparingly soluble: methanol, water Slightly soluble: ethanol, benzene chloroform	224.26	$C_{13}H_{12}N_4$	255	86.36	0.72
SY_{15}	5-amino 2-(4-nitrophenyl)-1*H*-benzimidazole	White colour solid	Freely soluble: Dimethylformamide and Dimethylsufoxide Soluble: chloroform Sparingly soluble: methanol, ethanol, acetone, water	254.24	$C_{13}H_{10}N_4O_2$	277	39.14	0.76

Table-2: Comparative physiochemical data of conventional and microwave assisted synthesized compounds

Compound	Conventional synthesis				Microwave synthesis			
	Reaction Time (h)	**% yield**	**Melting Point (˚ C)**	**R_f value Methanol : water (8:2)**	**Reaction Time (h)**	**% yield**	**Melting Point (˚ C)**	**R_f value Methanol : water (8:2)**
SY_1	6-8	37.94	162	0.79	2min 20sec	91.75	164	0.79
SY_2	6-8	39.94	112	0.84	1min 30sec	89.01	116	0.83
SY_3	6-8	54.02	114	0.86	1min 15sec	84.83	118	0.86
SY_4	2	35.37	248 (248-250)	0.30	6min 30sec	82.44	252	0.31
SY_5	4	31.38	254 (254-256)	0.58	8min 30sec	88.63	258	0.57

SCREENING OF ANTI-MICROBIAL ACTIVITY

6.4. a. Screening of anti-bacterial activity:

The synthesized compounds were evaluated for *in vitro* anti-bacterial activity against gram negative bacteria *Proteus vulgaris* NCTC 4635, *Klesibella pneumonia* ATCC 29655 and gram positive bacteria *Bacillus cereus* NL98, *Enterococcus faecium* ATCC 29212. These are the agents commonly causes urinary tract infection, nosocomial infection, biliary tract infection. The gram negative organism *Klesibella pneumonia* causes pneumonia, bronco pneumonia and bronchitis infection. The gram positive organisms *Bacillus cereus* and *Enterococcus faecium* cause endocarditis, bacteremia, meningitis and septicemia. As per the data obtained, it was confirmed that all the tested compounds possessed anti-bacterial activity against both gram positive and gram negative organisms. The SY_{11} showed the significant activity in following order *Proteus vulgaris* > *Enterococcus faecium* > *Bacillus cereus* > *Klesibella pneumonia*. The SY_{12} showed the siginificant activity in the following order *Bacillus cereus* > *Enterococcus faecium* > *Klesibella pneumonia* > *Proteus vulgaris.* The compound SY_{13} showed the significant activity in the following order *Proteus vulgaris* > *Klesibella pneumonia* > *Bacillus cereus* > *Enterococcus faecium.* The SY_{14} showed the significant activity in the following order *Klesibella pneumonia* > *Enterococcus faecium* > *Proteus vulgaris* > *Bacillus cereus*. The SY_{15} showed the significant activity in following order *Klesibella pneumonia* > *Bacillus cereus* > *Enterococcus faecium* > *Proteus vulgaris*. SY_{11} exhibited more potent activity against *Proteus vulgaris*. SY_{12} exhibited more potent activity towards *Bacillus cereus*. SY_{13} exhibited more potent activity towards *Proteus vulgaris*. SY_{14} exhibited more potent activity towards *Klesibella pneumonia*. SY_{15}

exhibited more potent activity against *Klesibella pneumonia*. However the anti-bacterial activity of all the synthesized compounds against the tested organism was found to be less than that of Ciprofloxacin a standard drug at tested dose level.

6.4. b. Screening of anti-fungal activity:

The synthesized compounds were evaluated *in-vitro* anti-fungal activity against two fungal organisms *Aspergillus niger* and *Aspergillus fumigatus*. These are the organisms causes serious lungs infection, bronchitis, aspergillosis. As per the data obtained, it was confirmed that all the tested compounds possessed anti-fungal activity. However, SY_{12} exhibited potent anti-fungal activity against both fungal organisms among the all synthesized compounds. However the anti-fungal activity of the SY_{12} against the tested organism was found to be less than that of anti-fungal drug ketoconazole at tested dose level.

Table 3: *In vitro* anti-bacterial activity of synthesized compounds by disc diffusion method

Microorganisms	Diameter of zone of inhibition in mm										
	SY_{11} (µg/disc)		SY_{12} (µg/disc)		SY_{13} (µg/disc)		SY_{14} (µg/disc)		SY_{15} (µg/disc)		Ciprofloxacin (µg/disc)
	25	100	25	100	25	100	25	100	25	100	100
Bacillus cereus	11	17	17	25.5	12	19	10	14.8	18	26	30
Proteus vulgaris	12	18.5	12	15	21	25	11	16.4	12	17	28
Klebsiella pneumonia	13	15	14	17	18	22.5	21	26	22	26.5	29
Enterococcus faecium	15	18	14	17.6	11	14	17	21	17	24	28

Table 4: *In vitro* anti-fungal activity of synthesized compounds by disc diffusion method

Microorganisms	Diameter of zone of inhibition in mm										
	SY_{11} (µg/disc)		SY_{12} (µg/disc)		SY_{13} (µg/disc)		SY_{14} (µg/disc)		SY_{15} (µg/disc)		Ketoconazole (µg/disc)
	25	100	25	100	25	100	25	100	25	100	100
Aspergillus niger	14	17.5	19	22	14	20	15	19.5	12	16.4	30
Aspergillus fumigatus	15	17	20	21	13	16	11	16.7	13	18	27

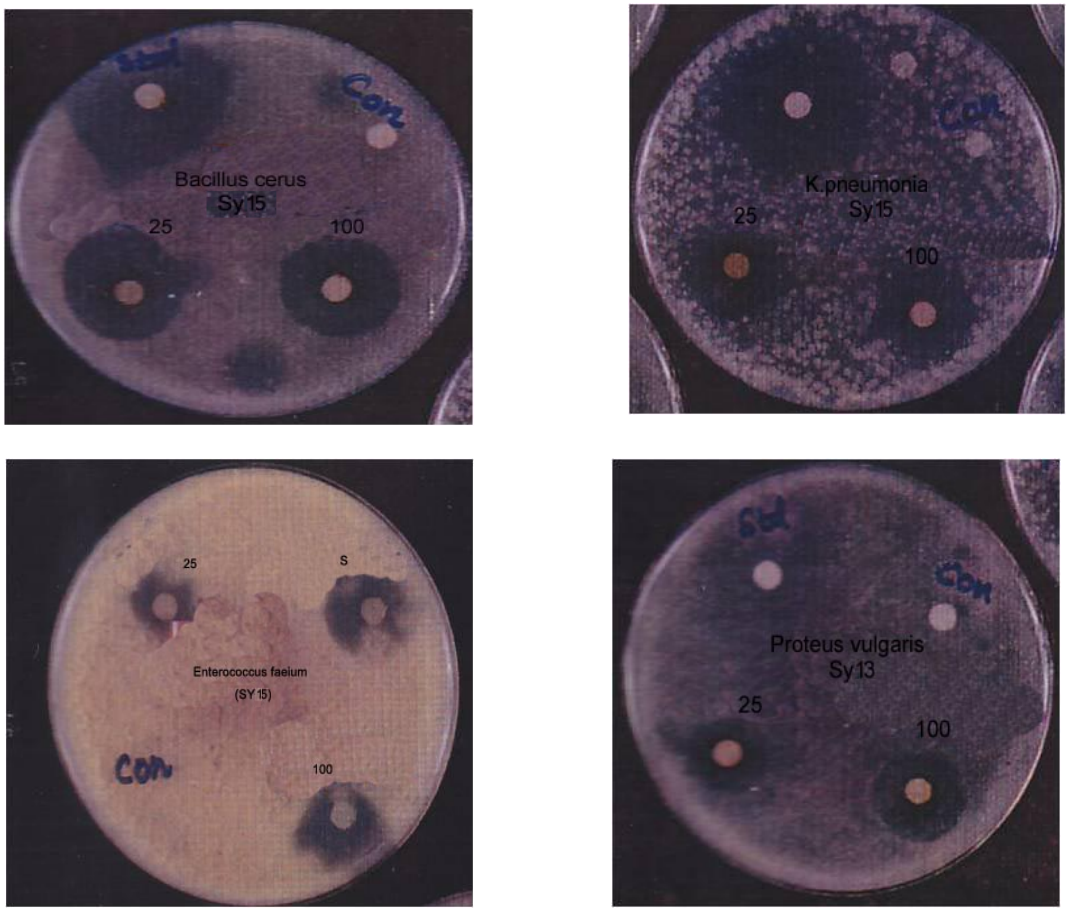


Fig-82: Anti-bacterial activity of synthesized compounds against tested organisms

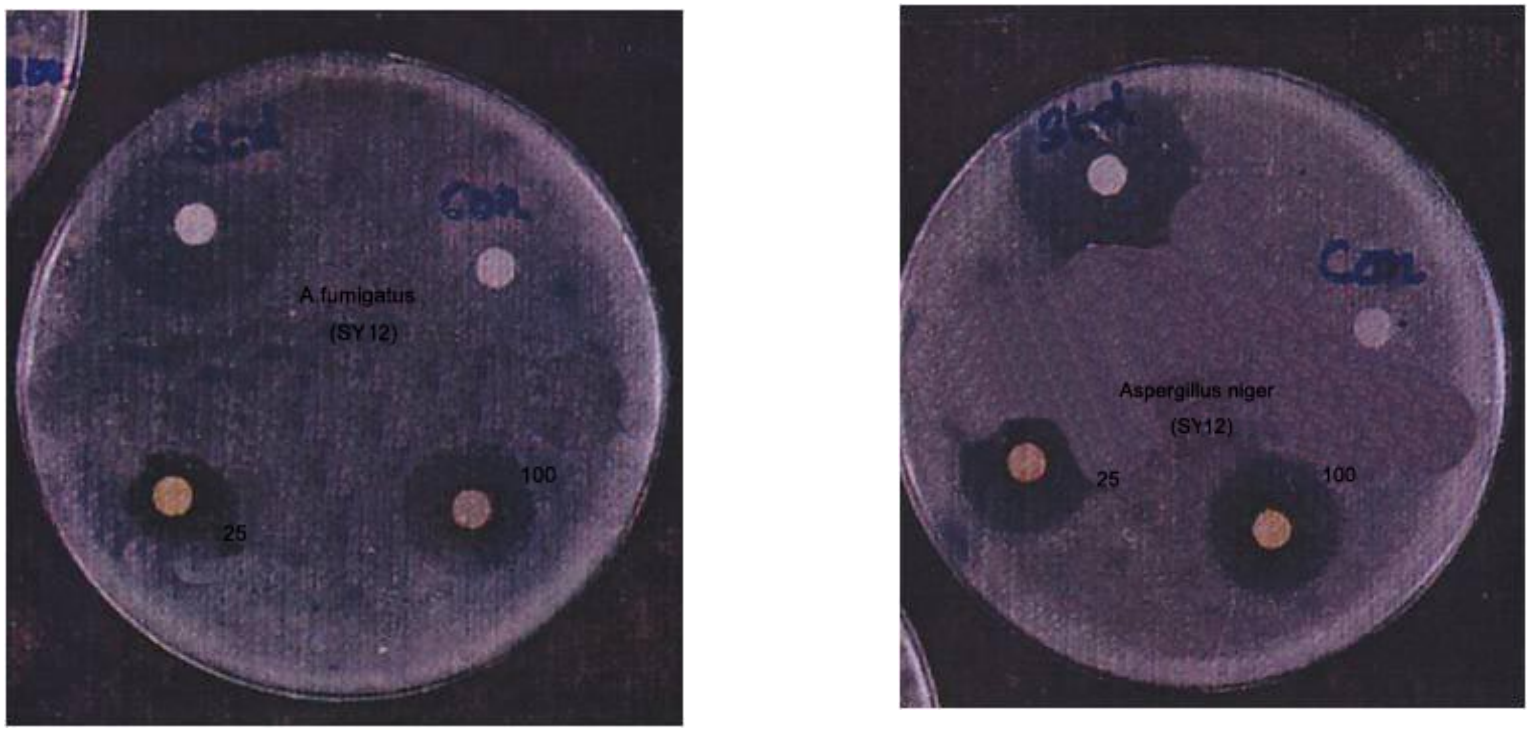


Fig-83: Anti-fungal activity of synthesized compounds against tested organisms

SUMMARY AND

CONCLUSION

7. SUMMARY AND CONCLUSION

In recent year attention has increasingly been given to the synthesis of benzimidazole derivatives as a source of new anti-microbial agents. The synthesis of novel benzimidazole derivatives remain a main focus of medicinal research. *(Vijaya B. Reddy, et al., 2009)*

In order to expand the group of benzimidazole derivatives, we synthesized several new benzimidaole ring containing compounds. The O-phenylene diamine reacted with appropriate carboxylic acid under harsh dehydrating reaction condition to give the corresponding benzimidazole in good yield by Phillips reaction. The same was repeated by using microwave irradiation to give the corresponding benzimidazole in excellent yields. Then, a series of 5- nitro substituted benzimidazole derivatives were synthesized using nitration reaction by using Conc. HNO_3 and Conc. H_2SO_4. Then 5-nitro substituted benzimidazoles were reduced by using a mixture of Zn/NaOH to give 5-amino substituted benzimidazoles. The purity of the synthesized compounds were checked by performing TLC (R_f) and determining melting points. Since our titled compounds were known to possess anti-microbial activity, the synthesized compounds were screened for their anti-bacterial and anti-fungal activity.

The structure of the synthesized compounds were established by spectral (IR, ^1H NMR and Mass) analysis data. The NH band (3463-3114 cm^{-1}) and NH proton signal (5.0 ppm) of 2-substituted benzimidazole in IR and ^1H NMR spectrum respectively in

the synthesized compounds, (SY_1-SY_5) confirmed the formation of benzimidazole nucleus. In SY_1, ^1H NMR spectrum showed a 2 proton singlet at δ 1.5 and δ 3.82 for 3 protons confirmed the presence of methane thiol group. In SY_2, multiplet at δ 3.12 for 1 proton and doublet at δ 1.29 for 6 protons indicated the formation of iso-propyl group.

In SY_3, two triplet at δ 2.55 and 0.96 for 5 protons and two multiplet at δ 1.62 and δ 1.37 for 4 protons indicated the presence of butyl group. In SY_4, two multiplet at δ 7.23-7.26 and δ 6.52 for 4 protons and a singlet at δ 4.0 for 2 protons indicated the presence of amino phenyl group. In the case of SY_5, two multiplet at δ 8.25 and δ 7.74 for 4 protons indicated the substitution of nitro phenyl group at C_2 of benzimidazole nucleus.

The presence of nitro group in SY_6-SY_{10} was ascertained from strong bands at 1584 -1532 cm^{-1} and 1345 cm^{-1} corresponding to asymmetric and symmetric O=N=O stretch respectively. Further a strong intensity signal at 1231-846 cm^{-1} was attributed to the C-N stretching for aromatic nitro compounds.

Spectrum of SY_6-SY_{10} in the aromatic region indicated that the three chemical environments at δ 8.63, 8.19 and 7.96, instead of two regions which were present in SY_1-SY_5. The maximal downfield one proton singlet at δ 8.63 was assigned to C-4 proton. The second proton (C-6), ortho to nitro group appeared at δ 8.19 as doublet (J = 4.5 Hz) due to ortho coupling with C-7 proton. The remaining C-7 proton was observed as a one proton doublet at δ 7.96 (J= 8.8 Hz) due to ortho coupling with C-6 proton.

The presence of primary amino group in SY_{11}-SY_{15} was ascertained from strong bands at 3500 cm^{-1} and 3400 cm^{-1} corresponding to asymmetric and symmetric H-N-H stretch respectively. Further a strong signal at 1648-1622 cm^{-1} was attributed to N-H bending for primary amino group.

In SY_{11} – $SY_{15,}$ a singlet at δ 3.48 for 2 protons indicated the presence of primary amino group.

In the mass spectrum of the synthesized compounds produced (M^+) Molecular ion peaks at 179.24, 175.23, 189.25, 224.26, 254.24 values for SY_{11}, SY_{12}, SY_{13}, SY_{14} and SY_{15} respectively corresponds to their molecular formulas. The predicted chemical structure of titled compounds was further supported by the fragmentation peaks.

All the synthesized compounds exhibited good activity against the studied set of microorganisms. Since a fewer species have been used in this study, it is warranted to screen these compounds with varied species and resistant strains. All the compounds showed very good anti- bacterial and anti-fungal activity even at less concentration. From the data, it is evident that the compound SY_{15} is a most potent candidate against anti-bacterial studies and compound SY_{12} is a much potent candidate for anti-fungal studies.

Even though, the antimicrobial activity of tested compounds was less than their standard compounds are ciprofloxacin (antibacterial) and ketoconazole (antifungal) in the present study. In future study, it could be increased (or) equalized by altering the

number of carbon atoms in side chain (or) introducing aromatic ring (or) substituted aromatic ring (or) heterocyclic ring (or) by introducing double bond in side chain in the 2^{nd} position of benzimidazole nucleus.

Further experiments were needed to elucidate their exact mechanism of activity. These results suggest that the tested derivatives of benzimidazoles have excellent scope for further development as commercial anti-bacterial and anti-fungal agents. In future study the activity of the compounds may be manipulated by introducing unsaturation or heterocyclic ring at C_2 of benzimidazole.

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ANNEXURE - I

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Research Article

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Synthesis and Biological Activity of Novel 2, 5-Disubstituted Benzimidazole Derivatives

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ABSTRACT

A new series of 2, 5 di-substituted benzimidazole derivatives have been synthesized. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral analysis and they were evaluated for their antibacterial; *Proteus vulgaris* (NCTC 4635), *Klesibella pneumonia* (ATCC 29655), *Bacillus cereus* (NL98), and *Enterococcus faecium* (ATCC 29212) and antifungal (*Aspergillus niger* and *Aspergillus fumigatus)* activities by disc diffusion method. All of the synthesized compounds showed good antibacterial and antifungal activity. However the antibacterial and antifungal activity of the synthesized compounds against the tested organisms was found to be less than that of respective standard drug at tested dose level.

Keywords: O-phenylene diamine, Benzimidazole, Antibacterial, Antifungal.

INTRODUCTION

Benzimidazole ring system known to be possess numerous antimicrobial [1-9], anti-inflammatory [9], anthelmintic [10], antiviral [11-13] and anti-tumour [14] properties. Therefore it was enabled that compounds containing benzimidazole nucleus would result in interesting of biological activities. In the present study 2-substituted benzimidazoles were synthesized by treating o-phenylene diamine with different carboxylic acids. Then they were subjected to nitration at room temperature to get 5-nitro 2-substituted benzimidazole derivatives. Finally they were reduced by using Zn/NaOH to get 5-amino 2-substituted benzimidazole derivatives. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral analysis. The newly synthesized final compounds were screened for their antibacterial and antifungal activity.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes on melting point apparatus (Sunbim, Guna enterprises) and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker-NMR 500 mHZ using MeOD and DMSO – d_6 as solvent. Mass spectra were recorded on JEOL GC mate mass spectrometer. The IR spectra of the synthesized compounds were recorded on Perkin-Elmer FT-IR spectrophotometer with KBr pellets. The UV spectra were recorded by using Double beam SHIMADZU 1700 UV spectrometer. The purity of the compounds was checked by TLC on pre-coated silica gel G plates by using methanol: water as a mobile phase and visualized in iodine vapour.

General Method for the Synthesis of 2-substituted benzimidazole derivatives [14-15]

O-phenylene diamine (0.25 mol) and appropriate carboxylic acid (0.34 mol) was heated on a water bath at 100°C for 6-8 h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using 10% sodium hydroxide solution. The crude benzimidazole was filtered at the pump, washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added and digested for 15 min. The solution was filtered while hot, cooled the filtrate to about 10°C. The pure product was filtered, washed with 25 ml of cold water and dried at 100°C.

General Method for the Synthesis of 5-nitro 2-substituted benzimidazole derivatives [16]

Conc. HNO_3 (7.5 ml) was placed in three necked round bottom flask fitted with a mechanical stirrer. The flask was immersed in ice cold water and added slowly conc. H_2SO_4 (7.5 ml) down the condenser with slow stirring. After the addition, 2-substituted benzimidazoles (0.028 mol) were added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35°C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over

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